



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

| | | |
|--|-----------|---|
| (51) International Patent Classification ⁵ : C12Q 1/02, G01N 33/68 | A1 | (11) International Publication Number: WO 93/05172 (43) International Publication Date: 18 March 1993 (18.03.93) |
| (21) International Application Number: PCT/US92/07359 (22) International Filing Date: 28 August 1992 (28.08.92) (30) Priority data: 752,861 30 August 1991 (30.08.91) US (71) Applicant: CREATIVE BIOMOLECULES, INC. [US/ US]; 35 South Street, Hopkinton, MA 01748 (US). (72) Inventors: SMART, John, E. ; 50 Meadow Brook Road, Weston, MA 02193 (US). OPPERMANN, Hermann ; 25 Summer Hill Road, Medway, MA 02053 (US). OZKAY- NAK, Engin ; 44 Purdue Drive, Milford, MA 01757 (US). KUBERASAMPATH, Thangavel ; 6 Spring Street, Medway, MA 02053 (US). RUEGER, David, C. ; 19 Downey Street, Hopkinton, MA 01748 (US). PANG, Roy, H., L. ; 15 Partridge Road, Etna, NH 03750 (US). COHEN, Charles, N. ; 98 Wintrop Street, Medway, MA 02053 (US). | | (74) Agent: KELLEY, Robin, D.; Testa, Hurwitz & Thibault, 53 State Street/Exchange Place, Boston, MA 02018-2809 (US). (81) Designated States: AU, CA, JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, SE). Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i> |
| (54) Title: MORPHOGENIC PROTEIN SCREENING METHOD (57) Abstract Disclosed is a method of screening candidate compounds for the ability to modulate the level of morphogenic protein in mammalian system. The method includes determining a parameter indicative of the level of production of a morphogenic in a cell culture known to produce the morphogen, incubating a candidate compound with the culture for a time sufficient to allow the compound to affect the production of the morphogenic protein, and then assaying the culture again to detect a change in the level of morphogenic protein production. | | |

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

| | | | | | |
|----|--------------------------|----|---------------------------------------|----|--------------------------|
| AT | Austria | FI | Finland | MN | Mongolia |
| AU | Australia | FR | France | MR | Mauritania |
| BB | Barbados | GA | Gabon | MW | Malawi |
| BE | Belgium | GB | United Kingdom | NL | Netherlands |
| BF | Burkina Faso | GN | Guinea | NO | Norway |
| BG | Bulgaria | GR | Greece | NZ | New Zealand |
| BJ | Benin | HU | Hungary | PL | Poland |
| BR | Brazil | IE | Ireland | PT | Portugal |
| CA | Canada | IT | Italy | RO | Romania |
| CF | Central African Republic | JP | Japan | RU | Russian Federation |
| CG | Congo | KP | Democratic People's Republic of Korea | SD | Sudan |
| CH | Switzerland | KR | Republic of Korea | SE | Sweden |
| CI | Côte d'Ivoire | LI | Liechtenstein | SK | Slovak Republic |
| CM | Cameroon | LK | Sri Lanka | SN | Senegal |
| CS | Czechoslovakia | LU | Luxembourg | SU | Soviet Union |
| CZ | Czech Republic | MC | Monaco | TD | Chad |
| DE | Germany | MG | Madagascar | TC | Togo |
| DK | Denmark | ML | Mali | UA | Ukraine |
| ES | Spain | | | US | United States of America |

1

MORPHOGENIC PROTEIN SCREENING METHOD

The invention relates to a method of screening drugs for the ability to modulate the level in mammals of proteins which can induce tissue morphogenesis and to methods of determining which animal tissue(s) and/or cell types within a tissue express a particular morphogenic protein.

Background of the Invention

Cell differentiation is the central characteristic of morphogenesis which initiates in the embryo, and continues to various degrees throughout the life of an organism in adult tissue repair and regeneration mechanisms. Members of the TGF- β superfamily include subfamilies of highly-related genes that now are suspected to play important roles in cell differentiation and morphogenesis during development and/or during adult life. For example, the *Drosophila* decapentaplegic gene product (DPP) has been implicated in formation of the dorsal-ventral axis in fruit flies; activins induce mesoderm and anterior structure formation in mammals; Müllerian inhibiting substance (MIS) may be required for male sex development in mammals; growth/differentiation factor-1 (GDF-1) has been implicated in nerve development and maintenance; other morphogenic proteins (BMP-2, -3, -4 and OP-1) induce bone formation.

The development and study of a bone induction model system has identified the developmental cascade of bone differentiation as consisting of chemotaxis of mesenchymal cells, proliferation of these progenitor cells, differentiation of cartilage, ossification and hypertrophy of this cartilaginous tissue, vascular invasion, bone formation, remodeling, and finally, marrow differentiation (Reddi (1981) Collagen Rel. Res. 1:209-206). This bone model system, which is studied in adult mammals, recapitulates the cascade of bone differentiation events that occur in formation of bone in the developing fetus. In other studies, the epithelium of the urinary bladder has been shown to induce new bone formation. Huggins (1931, Arch. Surg. 22:377-408) showed that new bone formation could be induced by surgical transplantation of urinary bladder epithelium onto the parietal fascia. Urist (1965, Science 150:893-899) demonstrated that implantation of demineralized bone segments resulted in endochondral bone formation. The latter study and observation suggested the existence of an osteogenic protein and that bovine diaphyseal bone was a source of enriched preparations of osteogenic protein (Sampath et al., J. Biol. Chem. 265:13198-13205, 1990; Urist, *ibid*; Reddi et al., Proc. Nat. Aca. Sci. 69:1601-1605, 1972; Sampath et al., Proc. Natl. Acad. Sci. 80:6591-6595, 1983). Proteins capable of inducing endochondral bone formation in mammals when implanted in association with a matrix now have been identified in a number of different mammalian species, as have the genes encoding these proteins, (see, for example, U.S. Patent No. 4,968,590; U.S.S.N. 315,342 filed February 23, 1989;

and U.S.S.N. 599,543, filed October 18, 1990). Human OP-1 DNA has been cloned from various cDNA and genomic libraries using a consensus probe (Ozkaynak et al., EMBO J. 9:2085-2093, 1990). Purified human recombinant OP-1, expressed in mammalian cells, has been shown to induce new bone formation in vivo. Like other members of the TGF- β superfamily, OP-1 is produced as a precursor, glycosylated, processed and secreted as a mature dimer. Mature OP-1 is cleaved at a maturation site following a sequence with the pattern of RXXR (Panganiban et al., Mol. Cell. Biol. 10:2669-2677, 1990).

The degree of morphogenesis in adult tissue varies among different tissues and depends on, among other factors, the degree of cell turnover in a given tissue. On this basis, tissues can be divided into three broad categories: 1) tissues with static cell populations such as nerve and skeletal muscle where there is little or no cell division and most of the cells formed during development persist throughout adult life and, therefore, possess little or no ability for normal regeneration after injury; 2) tissues containing conditionally renewing populations such as liver where there is generally little cell division but, in response to an appropriate stimulus or injury, cells can divide to produce daughters of the same differentiated cell type; and 3) tissues with permanently renewing populations including blood, bone, testes, and stratified squamous epithelia which are characterized by rapid and continuous cell turnover in the adult. Here, the terminally differentiated cells have a short life span and are replaced through

proliferati n f a distinct subpopulati n of cells, known as stem or progenitor cells.

It is an object of this invention to provide a method of screening compounds which, when administered to a given tissue from a given organism, cause an alteration in the level of morphogenic protein ("morphogen") produced by the tissue. Such compounds, when administered systemically, will result in altered systemic or local levels of morphogenic activity. This morphogenic activity includes the ability to induce proliferation and sequential differentiation of progenitor cells, and the ability to support and maintain the differentiated phenotype or sequence of phenotypes through the progression of events that results in the formation of normal adult tissue (including organ regeneration). Thus, broadly, the invention provides a key to development of additional modalities of therapies involving modulation of morphogenic protein production in animals or adult mammals, e.g., humans, and consequent correction of conditions involving pathologic alteration of the balance of tissue cell turnover. Another object of the invention is to provide methodologies for identifying or selecting a combination of compound(s) which may increase a progenitor cell population in a mammal, stimulate progenitor cells to differentiate in vivo or in vitro, maintain the differentiated phenotype or sequence of phenotypes of a tissue, induce tissue-specific growth in vivo, or replace diseased or damaged tissues or organs in vivo. Another object of the invention is to determine the tissue(s) or organ(s) of origin of a given morphogen. Another object of the

invention is to determine the specific cell type(s) within the tissue(s) or organ(s) of origin, or cell line(s) derived from the tissue(s), or organ(s) of origin, that is responsible for the synthesis and production of a given morphogen. These and other objects and features of the invention will be apparent from the description, drawing, and claims which follow.

Summary of th Invention

The invention features a method of screening candidate compounds for the ability to modulate the effective local or systemic concentration or level of morphogenic protein in an organism. The method is practiced by incubating one or more candidate compound(s) with cells from a test tissue type of an organism known to produce a given morphogen for a time sufficient to allow the compound(s) to affect the production, i.e., expression and/or secretion, of morphogen by the cells; and then assaying cells and the medium conditioned by the cells for a change in a parameter indicative of the level of production of the morphogenic protein. The procedure may be used to identify compounds showing promise as drugs for human use capable of increasing or decreasing morphogen production in vivo, thereby to correct or alleviate a diseased condition.

In a related aspect, the invention features a method of screening tissue(s) of an organism to assess whether or at what level cells of the tissue(s) produce a particular morphogen, thereby to determine a tissue(s) of origin of the morphogen. This permits selection of the tissue cell type to be used in the screening. As used herein, "tissue" refers to a group of cells which are naturally found associated, including an organ.

As an example of tissue(s) or organ(s) which produce high levels of morphogen relative to the level produced by other types of tissues, it has been discovered that OP-1, first found in bone tissue is produced at relatively high levels in cells derived

from renal, .g., kidney or bladder, r adr nal tissue; that GDF-1 is produced at relatively high levels in cells derived from nerve, e.g., brain tissue; that DPP is produced at relatively high levels in cells derived from one of the following drosophila tissues: dorsal ectoderm, epithelial imaginal disc, visceral mesoderm, or gut endoderm; that Vgr-1 is produced at relatively high levels in cells derived from mouse lung tissue; and that Vgl is produced at relatively high levels in cells derived from xenopus fetal endoderm tissue. In addition, BMP3 and CBMP2B transcripts have been identified in abundance in lung tissue. As used herein, "derived" means the cells are the cultured tissue itself, or are a cell line whose parent cells are the tissue itself.

Preferred methods for determining the level of or a change in the level of a morphogen in a cultured cell include using an antibody specific for the morphogen, e.g., in an immunoassay such as an ELISA or radioimmunoassay; and determining the level of nucleic acid, most particularly mRNA, encoding the morphogen using a nucleic acid probe that hybridizes under stringent conditions with the morphogen RNA, such as in an RNA dot blot analysis. Where a change in the presence and/or concentration of morphogen is being determined, it will be necessary to measure and compare the levels of morphogen in the presence and absence of the candidate compound. The nucleic acid probe may be a nucleotide sequence encoding the morphogen or a fragment large enough to hybridize specifically only to RNA encoding a specific morphogen under stringent conditions. As used herein, "stringent conditions" are

d find as conditions in which non-specific hybrids will be eluted but at which specific hybrids will be maintained, i.e., incubation at 0.1X SSC (15mM NaCl, 5mM Na citrate) at 50°C for 15 minutes.

Examples of morphogens whose levels may be determined according to the invention include OP-1, OP-2, GDF-1, Vgr-1, DPP, 60A CBMP2A, CBMP2B, BMP 2, 3, 4, 5, 6, or Vgl. Thus, if an immunoassay is used to indicate the presence and/or concentration of a morphogen, an antibody specific for one of these morphogens only, and which will not detect the presence of other morphogens, will be used. Similarly, if nucleic acid hybridization is used to indicate the level of RNA encoding the morphogen, a nucleotide probe specific for one of these morphogens only will be used under hybridization conditions such that the probe should not be capable of hybridizing with RNA encoding a different morphogen. A morphogen includes an active C-terminal core region, which includes at least six cysteine residues, and a region N-terminal to the C-terminal region that is relatively non-homologous to the equivalent N-terminal regions of other morphogens. In addition, the 3' noncoding region of the mRNA is unique to each morphogen. Thus, a nucleic acid probe encoding all or a portion of the sequences N-terminal to the C-terminal core region of a morphogen, or encoding all or a portion of the sequences C-terminal to or 3' to the core region of a morphogen may be used as a probe which detects mRNA encoding that morphogen only.

"Morphogenic proteins" or "morphogens", as used herein, include naturally-occurring osteogenic proteins

capable of inducing the full developmental cascade of bone formation, as well as polypeptide chains not normally associated with bone or bone formation, but sharing substantial sequence homology with osteogenic proteins. Such proteins, as well as DNA sequences encoding them, have been isolated and characterized for a number of different species. See, for example, U.S. Patent No. 4,968,590 and U.S. Patent Number. 5,011,691, U.S. application Serial Number 1989; 422,699, filed October 17, 1989, and 600,024 and 599,543, both filed October 18, 1990; Sampath et al., (1990) J. Biol. Chem. 265:13198-13205; Ozkaynak et al. (1990) EMBO J. 9:2085-2093; and Lee, Proc. Nat. Aca. Sci. 88:4250-4254 (1991), all of which are hereby incorporated by reference. Many of these proteins subsequently were discovered to have utility beyond bone morphogenesis. See, e.g., USSN 667,274 filed March 11, 1991. The mature forms of morphogens share substantial amino acid sequence homology, especially in the C-terminal core regions of the proteins. In particular, most of the proteins share a seven-cysteine skeleton in this region, in addition to other apparently required amino acids. Table II, infra, shows the amino acid sequence homologies for nine morphogens over the carboxy terminal 102 amino acids.

Among the morphogens useful in this invention are proteins originally identified as osteogenic proteins, such as the OP-1, OP-2 and CBMP2 proteins, as well as amino acid sequence-related proteins such as DPP (from *Drosophila*), Vgl (from *Xenopus*), Vgr-1 (from mouse, see U.S. 5,011,691 to Oppermann et al.), GDF-1 (from mouse, see Lee (1991) PNAS 88:4250-4254), all of which are

presented in Tabl II and Seq. ID Nos.5-14), and the recently identified 60A protein (from *Drosophila*, Seq. ID No. 24, see Wharton et al. (1991) PNAS 88:9214-9218.) The members of this family, which include members of the TGF- β super-family of proteins, share substantial amino acid sequence homology in their C-terminal regions. The proteins are translated as a precursor, having an N-terminal signal peptide sequence, typically less than about 30 residues, followed by a "pro" domain that is cleaved to yield the mature sequence. The signal peptide is cleaved rapidly upon translation, at a cleavage site that can be predicted in a given sequence using the method of Von Heijne ((1986) Nucleic Acids Research 14:4683-4691.) Table I, below, describes the various morphogens identified to date, including their nomenclature as used herein, their Seq. ID references, and publication sources for the amino acid sequences for the full length proteins not included in the Seq. Listing. The disclosure of these publications is incorporated herein by reference.

TABLE I

"OP-1" refers generically to the group of morphogenically active proteins expressed from part or all of a DNA sequence encoding OP-1 protein, including allelic and species variants thereof, e.g., human OP-1 ("hOP-1", Seq. ID No. 5, mature protein amino acid sequence), or mouse OP-1 ("mOP-1", Seq. ID No. 6, mature protein amino acid sequence.) The

conserved seven cysteine skeleton is defined by residues 38 to 139 of Seq. ID Nos. 5 and 6. The cDNA sequences and the amino acids encoding the full length proteins are provided in Seq. ID Nos. 16 and 17 (hOP1) and Seq. ID Nos. 18 and 19 (mOP1.) The mature proteins are defined by residues 293-431 (hOP1) and 292-430 (mOP1). The "pro" regions of the proteins, cleaved to yield the mature, morphogenically active proteins are defined essentially by residues 30-292 (hOP1) and residues 30-291 (mOP1).

"OP-2"

refers generically to the group of active proteins expressed from part or all of a DNA sequence encoding OP-2 protein, including allelic and species variants thereof, e.g., human OP-2 ("hOP-2", Seq. ID No. 7, mature protein amino acid sequence) or mouse OP-2 ("mOP-2", Seq. ID No. 8, mature protein amino acid sequence). The conserved seven cysteine skeleton is defined by residues 38 to 139 of Seq. ID Nos. 7 and 8. The cDNA sequences and the amino acids encoding the full length proteins are provided in Seq. ID Nos. 20 and 21 (hOP2) and Seq. ID Nos. 22 and 23 (mOP2.) The mature proteins are defined essentially by residues 264-402 (hOP2) and 261-399 (mOP2). The "pro" regions of the proteins, cleaved to yield

the mature, morphogenically active proteins likely are defined essentially by residues 18-263 (hOP2) and residues 18-260 (mOP2). (Another cleavage site also occurs 21 residues upstream for both OP-2 proteins.)

"CBMP2"

refers generically to the morphogenically active proteins expressed from a part or all of a DNA sequence encoding the CBMP2 proteins, including allelic and species variants thereof, e.g., human CBMP2A ("CBMP2A(fx)", Seq ID No. 9) or human CBMP2B DNA ("CBMP2B(fx)", Seq. ID No. 10). The amino acid sequence for the full length proteins, referred to in the literature as BMP2A and BMP2B, or BMP2 and BMP4, appear in Wozney, et al. (1988) Science 242:1528-1534. The pro domain for BMP2 (BMP2A) likely includes residues 25-248 or 25-282; the mature protein, residues 249-396 or 283-396. The pro domain for BMP4 (BMP2B) likely includes residues 25-256 or 25-292; the mature protein, residues 257-408 or 293-408.

"DPP(fx)"

refers to protein sequences encoded by the Drosophila DPP gene and defining the conserved seven cysteine skeleton (Seq. ID No. 11). The amino acid sequence for the full length protein appears in Padgett, et al (1987) Nature 325: 81-84. The pro

domain likely extends from the signal peptide cleavage site to residue 456; the mature protein likely is defined by residues 457-588.

"Vgl(fx)" refers to protein sequences encoded by the *Xenopus* Vgl gene and defining the conserved seven cysteine skeleton (Seq. ID No. 12). The amino acid sequence for the full length protein appears in Weeks (1987) Cell 51: 861-867. The pro domain likely extends from the signal peptide cleavage site to residue 246; the mature protein likely is defined by residues 247-360.

"Vgr-1(fx)" refers to protein sequences encoded by the murine Vgr-1 gene and defining the conserved seven cysteine skeleton (Seq. ID No. 13). The amino acid sequence for the full length protein appears in Lyons, et al, (1989) PNAS 86: 4554-4558. The pro domain likely extends from the signal peptide cleavage site to residue 299; the mature protein likely is defined by residues 300-438.

"GDF-1(fx)" refers to protein sequences encoded by the human GDF-1 gene and defining the conserved seven cysteine skeleton (Seq. ID No. 14). The cDNA and encoded amino sequence for the full length protein is

provided in Seq. ID. No. 32. The pro domain likely extends from the signal peptide cleavage site to residue 214; the mature protein likely is defined by residues 215-372.

"60A"

refers generically to the morphogenically active proteins expressed from part or all of a DNA sequence (from the Drosophila 60A gene) encoding the 60A proteins (see Seq. ID No. 24 wherein the cDNA and encoded amino acid sequence for the full length protein is provided). "60A(fx)" refers to the protein sequences defining the conserved seven cysteine skeleton (residues 354 to 455 of Seq. ID No. 24.) The pro domain likely extends from the signal peptide cleavage site to residue 324; the mature protein likely is defined by residues 325-455.

"BMP3(fx)"

refers to protein sequences encoded by the human BMP3 gene and defining the conserved seven cysteine skeleton (Seq. ID No. 26). The amino acid sequence for the full length protein appears in Wozney et al. (1988) Science 242: 1528-1534. The pro domain likely extends from the signal peptide cleavage site to residue 290; the mature protein likely is defined by residues 291-472.

"BMP5(fx)" refers to protein sequences encoded by the human BMP5 gene and defining the conserved seven cysteine skeleton (Seq. ID No. 27). The amino acid sequence for the full length protein appears in Celeste, et al. (1991) PNAS 87: 9843-9847. The pro domain likely extends from the signal peptide cleavage site to residue 316; the mature protein likely is defined by residues 317-454.

"BMP6(fx)" refers to protein sequences encoded by the human BMP6 gene and defining the conserved seven cysteine skeleton (Seq. ID No. 28). The amino acid sequence for the full length protein appears in Celeste, et al. (1990) PNAS 87: 9843-5847. The pro domain likely includes extends from the signal peptide cleavage site to residue 374; the mature sequence likely includes residues 375-513.

The OP-2 proteins have an additional cysteine residue in this region (e.g., see residue 41 of Seq. ID Nos. 7 and 8), in addition to the conserved cysteine skeleton in common with the other proteins in this family. The GDF-1 protein has a four amino acid insert within the conserved skeleton (residues 44-47 of Seq. ID No. 14) but this insert likely does not interfere with the relationship of the cysteines in the folded

structure. In addition, the CBMP2 proteins are missing one amino acid residue within the cysteine skeleton.

The morphogens are inactive when reduced, but are active as oxidized homodimers and when oxidized in combination with other morphogens of this invention. Thus, as defined herein, a morphogen is a dimeric protein comprising a pair of polypeptide chains, wherein each polypeptide chain comprises at least the C-terminal six cysteine skeleton defined by residues 43-139 of Seq. ID No. 5, including functionally equivalent arrangements of these cysteines (e.g., amino acid insertions or deletions which alter the linear arrangement of the cysteines in the sequence but not their relationship in the folded structure), such that, when the polypeptide chains are folded, the dimeric protein species comprising the pair of polypeptide chains has the appropriate three-dimensional structure, including the appropriate intra- and inter-chain disulfide bonds such that the protein is capable of acting as a morphogen as defined herein. Specifically, the morphogens generally are capable of the following biological functions in a morphogenically permissive environment: stimulating proliferation of progenitor cells; stimulating the differentiation of progenitor cells; stimulating the proliferation of differentiated cells; and supporting the growth and maintenance of differentiated cells, including the "redifferentiation" of transformed cells. In addition, it is also anticipated that these morphogens are capable of

inducing redifferentiation of committed cells under appropriate environmental conditions.

Morphogens useful in this invention comprise one of two species of generic amino acid sequences: Generic Sequence 1 (Seq. ID No. 1) or Generic Sequence 2 (Seq. ID No. 2); where each Xaa indicates one of the 20 naturally-occurring L-isomer, α -amino acids or a derivative thereof. Generic Sequence 1 comprises the conserved six cysteine skeleton and Generic Sequence 2 comprises the conserved six cysteine skeleton plus the additional cysteine identified in OP-2 (see residue 36, Seq. ID No. 2). In another preferred aspect, these sequences further comprise the following additional sequence at their N-terminus:

Cys Xaa Xaa Xaa Xaa (Seq. ID No. 15)
1 5

Preferred amino acid sequences within the foregoing generic sequences include: Generic Sequence 3 (Seq. ID No. 3), Generic Sequence 4 (Seq. ID No. 4), Generic Sequence 5 (Seq. ID No. 30) and Generic Sequence 6 (Seq. ID No. 31), listed below. These Generic Sequences accommodate the homologies shared among the various preferred members of this morphogen family identified in Table II, as well as the amino acid sequence variation among them. Specifically, Generic Sequences 3 and 4 are composite amino acid sequences of the following proteins presented in Table II and identified in Seq. ID Nos. 5-14: human OP-1 (hOP-1, Seq. ID Nos. 5 and 16-17), mouse OP-1 (mOP-1, Seq. ID

Nos. 6 and 18-19), human and mouse OP-2 (Seq. ID Nos. 7, 8, and 20-22), CBMP2A (Seq. ID No. 9), CBMP2B (Seq. ID No. 10), DPP (from *Drosophila*, Seq. ID No. 11), Vgl, (from *Xenopus*, Seq. ID No. 12), Vgr-1 (from mouse, Seq. ID No. 13), and GDF-1 (from mouse, Seq. ID No. 14.) The generic sequences include both the amino acid identity shared by the sequences in Table II, as well as alternative residues for the variable positions within the sequence. Note that these generic sequences allow for an additional cysteine at position 41 or 46 in Generic Sequences 3 or 4, respectively, providing an appropriate cysteine skeleton where inter- or intramolecular disulfide bonds can form, and contain certain critical amino acids which influence the tertiary structure of the proteins.

Generic Sequence 3

Leu Tyr Val Xaa Phe

1

5

Xaa Xaa Xaa Gly Trp Xaa Xaa Trp Xaa

10

Xaa Ala Pro Xaa Gly Xaa Xaa Ala

15

20

Xaa Tyr Cys Xaa Gly Xaa Cys Xaa

25

30

Xaa Pro Xaa Xaa Xaa Xaa Xaa

35

Xaa Xaa Xaa Asn His Ala Xaa Xaa

40

45

Xaa Xaa Leu Xaa Xaa Xaa Xaa

50

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys

55

60

Cys Xaa Pro Xaa Xaa Xaa Xaa Xaa

65

Xaa Xaa Xaa Leu Xaa Xaa Xaa

70

75

Xaa Xaa Xaa Xaa Val Xaa Leu Xaa

80

Xaa Xaa Xaa Xaa Met Xaa Val Xaa

85

90

Xaa Cys Gly Cys Xaa

95

wherein each Xaa is independently selected from a group of one or more specified amino acids defined as follows: "Res." means "residue" and Xaa at res.4 = (Ser, Asp or Glu); Xaa at res.6 = (Arg, Gln, Ser or Lys); Xaa at res.7 = (Asp or Glu); Xaa at res.8 = (Leu or Val); Xaa at res.11 = (Gln, Leu, Asp, His or Asn); Xaa at res.12 = (Asp, Arg or Asn); Xaa at res.14 = (Ile or Val); Xaa at res.15 = (Ile or Val); Xaa at res.18 = (Glu, Gln, Leu, Lys, Pro or Arg); Xaa at res.20 = (Tyr or Phe); Xaa at res.21 = (Ala, Ser, Asp, Met, His, Leu or Gln); Xaa at res.23 = (Tyr, Asn or Phe); Xaa at

res.26 = (Glu, His, Tyr, Asp or Gln); Xaa at res.28 = (Glu, Lys, Asp or Gln); Xaa at res.30 = (Ala, Ser, Pro or Gln); Xaa at res.31 = (Phe, Leu or Tyr); Xaa at res.33 = (Leu or Val); Xaa at res.34 = (Asn, Asp, Ala or Thr); Xaa at res.35 = (Ser, Asp, Glu, Leu or Ala); Xaa at res.36 = (Tyr, Cys, His, Ser or Ile); Xaa at res.37 = (Met, Phe, Gly or Leu); Xaa at res.38 = (Asn or Ser); Xaa at res.39 = (Ala, Ser or Gly); Xaa at res.40 = (Thr, Leu or Ser); Xaa at res.44 = (Ile or Val); Xaa at res.45 = (Val or Leu); Xaa at res.46 = (Gln or Arg); Xaa at res.47 = (Thr, Ala or Ser); Xaa at res.49 = (Val or Met); Xaa at res.50 = (His or Asn); Xaa at res.51 = (Phe, Leu, Asn, Ser, Ala or Val); Xaa at res.52 = (Ile, Met, Asn, Ala or Val); Xaa at res.53 = (Asn, Lys, Ala or Glu); Xaa at res.54 = (Pro or Ser); Xaa at res.55 = (Glu, Asp, Asn, or Gly); Xaa at res.56 = (Thr, Ala, Val, Lys, Asp, Tyr, Ser or Ala); Xaa at res.57 = (Val, Ala or Ile); Xaa at res.58 = (Pro or Asp); Xaa at res.59 = (Lys or Leu); Xaa at res.60 = (Pro or Ala); Xaa at res.63 = (Ala or Val); Xaa at res.65 = (Thr or Ala); Xaa at res.66 = (Gln, Lys, Arg or Glu); Xaa at res.67 = (Leu, Met or Val); Xaa at res.68 = (Asn, Ser or Asp); Xaa at res.69 = (Ala, Pro or Ser); Xaa at res.70 = (Ile, Thr or Val); Xaa at res.71 = (Ser or Ala); Xaa at res.72 = (Val or Met); Xaa at res.74 = (Tyr or Phe); Xaa at res.75 = (Phe, Tyr or Leu); Xaa at res.76 = (Asp or Asn); Xaa at res.77 = (Asp, Glu, Asn or Ser); Xaa at res.78 = (Ser, Gln, Asn or Tyr); Xaa at res.79 = (Ser, Asn, Asp or Glu); Xaa at res.80 = (Asn, Thr or Lys); Xaa at res.82 = (Ile or Val); Xaa at res.84 = (Lys or Arg); Xaa at res.85 = (Lys, Asn, Gln or His); Xaa at res.86 = (Tyr or His);

Xaa at r s.87 = (Arg, Gln or Glu); Xaa at res.88 = (Asn, Glu or Asp); Xaa at res.90 = (Val, Thr or Ala); Xaa at res.92 = (Arg, Lys, Val, Asp or Glu); Xaa at res.93 = (Ala, Gly or Glu); and Xaa at res.97 = (His or Arg);

Generic Sequence 4

| | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|----|--|
| Cys | Xaa | Xaa | Xaa | Xaa | Leu | Tyr | Val | Xaa | Phe | | |
| 1 | | | | | 5 | | | | 10 | | |
| Xaa | Xaa | Xaa | Gly | Trp | Xaa | Xaa | Trp | Xaa | | | |
| | | | | | | | | | | 15 | |
| Xaa | Ala | Pro | Xaa | Gly | Xaa | Xaa | Ala | | | | |
| 20 | | | | | | | 25 | | | | |
| Xaa | Tyr | Cys | Xaa | Gly | Xaa | Cys | Xaa | | | | |
| | | | 30 | | | | 35 | | | | |
| Xaa | Pro | Xaa | Xaa | Xaa | Xaa | Xaa | Xaa | | | | |
| | | | | | | | | | | 40 | |
| Xaa | Xaa | Xaa | Asn | His | Ala | Xaa | Xaa | | | | |
| | | | 45 | | | | 50 | | | | |
| Xaa | Xaa | Leu | Xaa | Xaa | Xaa | Xaa | Xaa | | | | |
| | | | | | | | | | | 55 | |
| Xaa | Xaa | Xaa | Xaa | Xaa | Xaa | Xaa | Xaa | Cys | | | |
| | | | 60 | | | | 65 | | | | |
| Cys | Xaa | Pro | Xaa | Xaa | Xaa | Xaa | Xaa | Xaa | | | |
| | | | | | | | | | | 70 | |
| Xaa | Xaa | Xaa | Leu | Xaa | Xaa | Xaa | | | | | |
| 75 | | | | | | | 80 | | | | |
| Xaa | Xaa | Xaa | Xaa | Val | Xaa | Leu | Xaa | | | | |
| | | | | | | | | | | 85 | |
| Xaa | Xaa | Xaa | Xaa | Met | Xaa | Val | Xaa | | | | |
| 90 | | | | | | | 95 | | | | |

Xaa Cys Gly Cys Xaa

100

wherein each Xaa is independently selected from a group of one or more specified amino acids as defined by the following: "Res." means "residue" and Xaa at res.2 = (Lys or Arg); Xaa at res.3 = (Lys or Arg); Xaa at res.4 = (His or Arg); Xaa at res.5 = (Glu, Ser, His, Gly, Arg or Pro); Xaa at res.9 = (Ser, Asp or Glu); Xaa at res.11 = (Arg, Gln, Ser or Lys); Xaa at res.12 = (Asp or Glu); Xaa at res.13 = (Leu or Val); Xaa at res.16 = (Gln, Leu, Asp, His or Asn); Xaa at res.17 = (Asp, Arg, or Asn); Xaa at res.19 = (Ile or Val); Xaa at res.20 = (Ile or Val); Xaa at res.23 = (Glu, Gln, Leu, Lys, Pro or Arg); Xaa at res.25 = (Tyr or Phe); Xaa at res.26 = (Ala, Ser, Asp, Met, His, Leu, or Gln); Xaa at res.28 = (Tyr, Asn or Phe); Xaa at res.31 = (Glu, His, Tyr, Asp or Gln); Xaa at res.33 = Glu, Lys, Asp or Gln; Xaa at res.35 = (Ala, Ser or Pro); Xaa at res.36 = (Phe, Leu or Tyr); Xaa at res.38 = (Leu or Val); Xaa at res.39 = (Asn, Asp, Ala or Thr); Xaa at res.40 = (Ser, Asp, Glu, Leu or Ala); Xaa at res.41 = (Tyr, Cys, His, Ser or Ile); Xaa at res.42 = (Met, Phe, Gly or Leu); Xaa at res.44 = (Ala, Ser or Gly); Xaa at res.45 = (Thr, Leu or Ser); Xaa at res.49 = (Ile or Val); Xaa at res.50 = (Val or Leu); Xaa at res.51 = (Gln or Arg); Xaa at res.52 = (Thr, Ala or Ser); Xaa at res.54 = (Val or Met); Xaa at res.55 = (His or Asn); Xaa at res.56 = (Phe, Leu, Asn, Ser, Ala or Val); Xaa at res.57 = (Ile, Met, Asn, Ala or Val); Xaa at res.58 = (Asn, Lys, Ala or Glu); Xaa at res.59 = (Pro or Ser); Xaa at res.60 = (Glu, Asp, or Gly); Xaa at res.61 = (Thr, Ala, Val, Lys, Asp, Tyr, Ser or Ala); Xaa at res.62 = (Val, Ala

or Ile); Xaa at res.63 = (Pr or Asp); Xaa at res.64 = (Lys or Leu); Xaa at res.65 = (Pro or Ala); Xaa at res.68 = (Ala or Val); Xaa at res.70 = (Thr or Ala); Xaa at res.71 = (Gln, Lys, Arg or Glu); Xaa at res.72 = (Leu, Met or Val); Xaa at res.73 = (Asn, Ser or Asp); Xaa at res.74 = (Ala, Pro or Ser); Xaa at res.75 = (Ile, Thr or Val); Xaa at res.76 = (Ser or Ala); Xaa at res.77 = (Val or Met); Xaa at res.79 = (Tyr or Phe); Xaa at res.80 = (Phe, Tyr or Leu); Xaa at res.81 = (Asp or Asn); Xaa at res.82 = (Asp, Glu, Asn or Ser); Xaa at res.83 = (Ser, Gln, Asn or Tyr); Xaa at res.84 = (Ser, Asn, Asp or Glu); Xaa at res.85 = (Asn, Thr or Lys); Xaa at res.87 = (Ile or Val); Xaa at res.89 = (Lys or Arg); Xaa at res.90 = (Lys, Asn, Gln or His); Xaa at res.91 = (Tyr or His); Xaa at res.92 = (Arg, Gln or Glu); Xaa at res.93 = (Asn, Glu or Asp); Xaa at res.95 = (Val, Thr or Ala); Xaa at res.97 = (Arg, Lys, Val, Asp or Glu); Xaa at res.98 = (Ala, Gly or Glu); and Xaa at res.102 = (His or Arg).

Similarly, Generic Sequence 5 (Seq. ID No. 30) and Generic Sequence 6 (Seq. ID No. 31) accommodate the homologies shared among all the morphogen protein family members identified in Table II. Specifically, Generic Sequences 5 and 6 are composite amino acid sequences of human OP-1 (hOP-1, Seq. ID Nos. 5 and 16-17), mouse OP-1 (mOP-1, Seq. ID Nos. 6 and 18-19), human and mouse OP-2 (Seq. ID Nos. 7, 8, and 20-22), CBMP2A (Seq. ID No. 9), CBMP2B (Seq. ID No. 10), DPP (from *Drosophila*, Seq. ID No. 11), Vgl, (from *Xenopus*, Seq. ID No. 12), Vgr-1 (from mouse, Seq. ID No. 13), and GDF-1 (from mouse, Seq. ID No. 14), human BMP3

(Seq. ID No. 26), human BMP5 (Seq. ID No. 27), human BMP6 (Seq. ID No. 28) and 60(A) (from *Drosophila*, Seq. ID Nos. 24-25). The generic sequences include both the amino acid identity shared by these sequences in the C-terminal domain, defined by the six and seven cysteine skeletons (Generic Sequences 5 and 6, respectively), as well as alternative residues for the variable positions within the sequence. As for Generic Sequences 3 and 4, Generic Sequences 5 and 6 allow for an additional cysteine at position 41 (Generic Sequence 5) or position 46 (Generic Sequence 6), providing an appropriate cysteine skeleton where inter- or intramolecular disulfide bonds can form, and containing certain critical amino acids which influence the tertiary structure of the proteins.

Generic Sequence 5

```

Leu Xaa Xaa Xaa Phe
 1                      5
Xaa Xaa Xaa Gly Trp Xaa Xaa Trp Xaa
                10
Xaa Xaa Pro Xaa Xaa Xaa Xaa Ala
 15                      20
Xaa Tyr Cys Xaa Gly Xaa Cys Xaa
                25                      30
Xaa Pro Xaa Xaa Xaa Xaa Xaa
                35

```

Xaa Xaa Xaa Asn His Ala Xaa Xaa

40

45

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa

50

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys

55

60

Cys Xaa Pro Xaa Xaa Xaa Xaa Xaa

65

Xaa Xaa Xaa Leu Xaa Xaa Xaa

70

75

Xaa Xaa Xaa Xaa Val Xaa Leu Xaa

80

Xaa Xaa Xaa Xaa Met Xaa Val Xaa

85

90

Xaa Cys Xaa Cys Xaa

95

wherein each Xaa is independently selected from a group of one or more specified amino acids defined as follows: "Res." means "residue" and Xaa at res.2 = (Tyr or Lys); Xaa at res.3 = Val or Ile); Xaa at res.4 = (Ser, Asp or Glu); Xaa at res.6 = (Arg, Gln, Ser, Lys or Ala); Xaa at res.7 = (Asp, Glu or Lys); Xaa at res.8 = (Leu, Val or Ile); Xaa at res.11 = (Gln, Leu, Asp, His, Asn or Ser); Xaa at res.12 = (Asp, Arg, Asn or Glu); Xaa at res.14 = (Ile or Val); Xaa at res.15 = (Ile or Val); Xaa at res.16 (Ala or Ser); Xaa at res.18 = (Glu, Gln, Leu, Lys, Pro or Arg); Xaa at res.19 =

(Gly or Ser); Xaa at res.20 = (Tyr or Phe); Xaa at res.21 = (Ala, Ser, Asp, Met, His, Gln, Leu or Gly); Xaa at res.23 = (Tyr, Asn or Phe); Xaa at res.26 = (Glu, His, Tyr, Asp, Gln or Ser); Xaa at res.28 = (Glu, Lys, Asp, Gln or Ala); Xaa at res.30 = (Ala, Ser, Pro, Gln or Asn); Xaa at res.31 = (Phe, Leu or Tyr); Xaa at res.33 = (Leu, Val or Met); Xaa at res.34 = (Asn, Asp, Ala, Thr or Pro); Xaa at res.35 = (Ser, Asp, Glu, Leu, Ala or Lys); Xaa at res.36 = (Tyr, Cys, His, Ser or Ile); Xaa at res.37 = (Met, Phe, Gly or Leu); Xaa at res.38 = (Asn, Ser or Lys); Xaa at res.39 = (Ala, Ser, Gly or Pro); Xaa at res.40 = (Thr, Leu or Ser); Xaa at res.44 = (Ile, Val or Thr); Xaa at res.45 = (Val, Leu or Ile); Xaa at res.46 = (Gln or Arg); Xaa at res.47 = (Thr, Ala or Ser); Xaa at res.48 = (Leu or Ile); Xaa at res.49 = (Val or Met); Xaa at res.50 = (His, Asn or Arg); Xaa at res.51 = (Phe, Leu, Asn, Ser, Ala or Val); Xaa at res.52 = (Ile, Met, Asn, Ala, Val or Leu); Xaa at res.53 = (Asn, Lys, Ala, Glu, Gly or Phe); Xaa at res.54 = (Pro, Ser or Val); Xaa at res.55 = (Glu, Asp, Asn, Gly, Val or Lys); Xaa at res.56 = (Thr, Ala, Val, Lys, Asp, Tyr, Ser, Ala, Pro or His); Xaa at res.57 = (Val, Ala or Ile); Xaa at res.58 = (Pro or Asp); Xaa at res.59 = (Lys, Leu or Glu); Xaa at res.60 = (Pro or Ala); Xaa at res.63 = (Ala or Val); Xaa at res.65 = (Thr, Ala or Glu); Xaa at res.66 = (Gln, Lys, Arg or Glu); Xaa at res.67 = (Leu, Met or Val); Xaa at res.68 = (Asn, Ser, Asp or Gly); Xaa at res.69 = (Ala, Pro or Ser); Xaa at res.70 = (Ile, Thr, Val or Leu); Xaa at res.71 = (Ser, Ala or Pro); Xaa at res.72 = (Val, Met or Ile); Xaa at res.74 = (Tyr or Phe); Xaa at res.75 = (Phe, Tyr, Leu or His); Xaa at res.76 = (Asp, Asn or

Leu); Xaa at res.77 = (Asp, Glu, Asn or Ser); Xaa at res.78 = (Ser, Gln, Asn, Tyr or Asp); Xaa at res.79 = (Ser, Asn, Asp, Glu or Lys); Xaa at res.80 = (Asn, Thr or Lys); Xaa at res.82 = (Ile, Val or Asn); Xaa at res.84 = (Lys or Arg); Xaa at res.85 = (Lys, Asn, Gln, His or Val); Xaa at res.86 = (Tyr or His); Xaa at res.87 = (Arg, Gln, Glu or Pro); Xaa at res.88 = (Asn, Glu or Asp); Xaa at res.90 = (Val, Thr, Ala or Ile); Xaa at res.92 = (Arg, Lys, Val, Asp or Glu); Xaa at res.93 = (Ala, Gly, Glu or Ser); Xaa at res.95 = (Gly or Ala) and Xaa at res.97 = (His or Arg).

Generic Sequence 6

```

Cys Xaa Xaa Xaa Xaa Leu Xaa Xaa Xaa Phe
 1                      5                      10
Xaa Xaa Xaa Gly Trp Xaa Xaa Trp Xaa
                      15
Xaa Xaa Pro Xaa Xaa Xaa Xaa Ala
 20                      25
Xaa Tyr Cys Xaa Gly Xaa Cys Xaa
                      30                      35
Xaa Pro Xaa Xaa Xaa Xaa Xaa
                      40
Xaa Xaa Xaa Asn His Ala Xaa Xaa
                      45                      50
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
                      55
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
 60                      65
Cys Xaa Pro Xaa Xaa Xaa Xaa Xaa
                      70

```

Xaa Xaa Xaa Leu Xaa Xaa Xaa
75 80
Xaa Xaa Xaa Xaa Val Xaa Leu Xaa
85
Xaa Xaa Xaa Xaa Met Xaa Val Xaa
90 95
Xaa Cys Xaa Cys Xaa
100

wherein each Xaa is independently selected from a group of one or more specified amino acids as defined by the following: "Res." means "residue" and Xaa at res.2 = (Lys, Arg, Ala or Gln); Xaa at res.3 = (Lys, Arg or Met); Xaa at res.4 = (His, Arg or Gln); Xaa at res.5 = (Glu, Ser, His, Gly, Arg, Pro, Thr, or Tyr); Xaa at res.7 = (Tyr or Lys); Xaa at res.8 = (Val or Ile); Xaa at res.9 = (Ser, Asp or Glu); Xaa at res.11 = (Arg, Gln, Ser, Lys or Ala); Xaa at res.12 = (Asp, Glu, or Lys); Xaa at res.13 = (Leu, Val or Ile); Xaa at res.16 = (Gln, Leu, Asp, His, Asn or Ser); Xaa at res.17 = (Asp, Arg, Asn or Glu); Xaa at res.19 = (Ile or Val); Xaa at res.20 = (Ile or Val); Xaa at res.21 = (Ala or Ser); Xaa at res.23 = (Glu, Gln, Leu, Lys, Pro or Arg); Xaa at res.24 = (Gly or Ser); Xaa at res.25 = (Tyr or Phe); Xaa at res.26 = (Ala, Ser, Asp, Met, His, Gln, Leu, or Gly); Xaa at res.28 = (Tyr, Asn or Phe); Xaa at res.31 = (Glu, His, Tyr, Asp, Gln or Ser); Xaa at res.33 = Glu, Lys, Asp, Gln or Ala); Xaa at res.35 = (Ala, Ser, Pro, Gln or Asn); Xaa at res.36 = (Phe, Leu or Tyr); Xaa at res.38 = (Leu, Val or Met); Xaa at res.39 = (Asn, Asp, Ala, Thr or Pro); Xaa at res.40 = (Ser, Asp, Glu, Leu, Ala or Lys); Xaa at res.41 = (Tyr,

Cys, His, Ser or Il); Xaa at res.42 = (Met, Phe, Gly or Leu); Xaa at res.43 = (Asn, Ser or Lys); Xaa at res.44 = (Ala, Ser, Gly or Pro); Xaa at res.45 = (Thr, Leu or Ser); Xaa at res.49 = (Ile, Val or Thr); Xaa at res.50 = (Val, Leu or Ile); Xaa at res.51 = (Gln or Arg); Xaa at res.52 = (Thr, Ala or Ser); Xaa at res.53 = (Leu or Ile); Xaa at res.54 = (Val or Met); Xaa at res.55 = (His, Asn or Arg); Xaa at res.56 = (Phe, Leu, Asn, Ser, Ala or Val); Xaa at res.57 = (Ile, Met, Asn, Ala, Val or Leu); Xaa at res.58 = (Asn, Lys, Ala, Glu, Gly or Phe); Xaa at res.59 = (Pro, Ser or Val); Xaa at res.60 = (Glu, Asp, Gly, Val or Lys); Xaa at res.61 = (Thr, Ala, Val, Lys, Asp, Tyr, Ser, Ala, Pro or His); Xaa at res.62 = (Val, Ala or Ile); Xaa at res.63 = (Pro or Asp); Xaa at res.64 = (Lys, Leu or Glu); Xaa at res.65 = (Pro or Ala); Xaa at res.68 = (Ala or Val); Xaa at res.70 = (Thr, Ala or Glu); Xaa at res.71 = (Gln, Lys, Arg or Glu); Xaa at res.72 = (Leu, Met or Val); Xaa at res.73 = (Asn, Ser, Asp or Gly); Xaa at res.74 = (Ala, Pro or Ser); Xaa at res.75 = (Ile, Thr, Val or Leu); Xaa at res.76 = (Ser, Ala or Pro); Xaa at res.77 = (Val, Met or Ile); Xaa at res.79 = (Tyr or Phe); Xaa at res.80 = (Phe, Tyr, Leu or His); Xaa at res.81 = (Asp, Asn or Leu); Xaa at res.82 = (Asp, Glu, Asn or Ser); Xaa at res.83 = (Ser, Gln, Asn, Tyr or Asp); Xaa at res.84 = (Ser, Asn, Asp, Glu or Lys); Xaa at res.85 = (Asn, Thr or Lys); Xaa at res.87 = (Ile, Val or Asn); Xaa at res.89 = (Lys or Arg); Xaa at res.90 = (Lys, Asn, Gln, His or Val); Xaa at res.91 = (Tyr or His); Xaa at res.92 = (Arg, Gln, Glu or Pro); Xaa at res.93 = (Asn, Glu or Asp); Xaa at res.95 = (Val, Thr, Ala or Ile); Xaa at res.97 = (Arg, Lys, Val,

Asp or Glu); Xaa at res.98 = (Ala, Gly, Glu or Ser); Xaa at res.100 = (Gly or Ala); and Xaa at res.102 = (His or Arg).

Particularly useful sequences for use as morphogens in this invention include the C-terminal domains, e.g., the C-terminal 96-102 amino acid residues of Vgl, Vgr-1, DPP, OP-1, OP-2, CBMP-2A, CBMP-2B, GDF-1 (see Table II, below, and Seq. ID Nos. 5-14), as well as proteins comprising the C-terminal domains of 60A, BMP3, BMP5 and BMP6 (see Seq. ID Nos. 24-28), all of which include at least the conserved six or seven cysteine skeleton. In addition, biosynthetic constructs designed from the generic sequences, such as COP-1, 3-5, 7, 16, disclosed in U.S. Pat. No. 5,011,691, also are useful. Other sequences include the inhibins/activin proteins (see, for example, U.S. Pat. Nos. 4,968,590 and 5,011,691). Accordingly, other useful sequences are those sharing at least 70% amino acid sequence homology or "similarity", and preferably 80% homology or similarity with any of the sequences above. These are anticipated to include allelic and species variants and mutants, and biosynthetic muteins, as well as novel members of this morphogenic family of proteins. Particularly envisioned in the family of related proteins are those proteins exhibiting morphogenic activity and wherein the amino acid changes from the preferred sequences include conservative changes, e.g., those as defined by Dayoff et al., Atlas of Protein Sequence and Structure; vol. 5, Suppl. 3, pp. 345-362, (M.O. Dayoff, ed., Nat'l BioMed. Research Fdn., Washington, D.C. 1979). As used

herein, potentially useful sequences are aligned with a known morphogen sequence using the method of Needleman et al. ((1970) J.Mol.Biol. 48:443-453) and identities calculated by the Align program (DNASTar, Inc.). "Homology" or "similarity" as used herein includes allowed conservative changes as defined by Dayoff et al.

Morphogen sequences which are detectable according to the methods of the invention include but are not limited to those having greater than 60% identity, preferably greater than 65% identity, with the amino acid sequence defining the conserved six cysteine skeleton of hOP1 (e.g., residues 43-139 of Seq. ID No. 5). These most preferred sequences include both allelic and species variants of the OP-1 and OP-2 proteins, including the Drosophila 60A protein. Accordingly, morphogens which are detectable according to the invention include active proteins comprising species of polypeptide chains having the generic amino acid sequence herein referred to as "OPX", which accommodates the homologies between the various identified species of OP1 and OP2 (Seq. ID No. 29).

The morphogens detectable in the methods of this invention include proteins comprising any of the polypeptide chains described above, whether isolated from naturally-occurring sources, or produced by recombinant DNA or other synthetic techniques, and includes allelic and species variants of these proteins, naturally-occurring or biosynthetic mutants thereof, chimeric variants containing a domain(s) or

region(s) of one family member functionally arranged with another domain(s) or regions(s) of a second family member, as well as various truncated and fusion constructs. Deletion or insertion or addition mutants also are envisioned to be active, including those which may alter the conserved C-terminal cysteine skeleton, provided that the alteration does not functionally disrupt the relationship of these cysteines in the folded structure. Accordingly, such active forms are considered the equivalent of the specifically described constructs disclosed herein. The proteins may include forms having varying glycosylation patterns, varying N-termini, a family of related proteins having regions of amino acid sequence homology, and active truncated or mutated forms of native or biosynthetic proteins, produced by expression of recombinant DNA in host cells.

The morphogenic proteins can be expressed from intact or truncated cDNA or from synthetic DNAs in procaryotic or eucaryotic host cells, and purified, cleaved, refolded, and dimerized to form morphogenically active compositions. Currently preferred host cells include E. coli or mammalian cells, such as CHO, COS or BSC cells. A detailed description of the morphogens detectable according to the methods of this invention is disclosed in copending US patent application Serial Nos. 752,764, filed August 30, 1991, and 667,274, filed March 11, 1991, the disclosure of which are incorporated herein by reference.

The screening method of the invention provides a simple method of determining a change in the level of morphogenic protein as a result of exposure of cultured cells to one or more compound(s). The level of a morphogenic protein in a given cell culture, or a change in that level resulting from exposure to one or more compound(s) indicates that direct application of the compound modulates the level of the morphogen expressed by the cultured cells. If, for example, a compound upregulated the production of OP-1 by a kidney cell line, it would then be desirable to test systemic administration of this compound in an animal model to determine if it upregulated the production of OP-1 in vivo. If this compound did upregulate the endogenous circulating levels of OP-1, it would be consistent with administration of the compound systemically for the purpose of correcting bone metabolism diseases such as osteoporosis. The level of morphogen in the body may be a result of a wide range of physical conditions, e.g., tissue degeneration such as occurs in diseases including arthritis, emphysema, osteoporosis, kidney diseases, lung diseases, cardiomyopathy, and cirrhosis of the liver. The level of morphogens in the body may also occur as a result of the normal process of aging. A compound selected by the screening method of the invention as, for example, one which increases the level of morphogen in a tissue, may be consistent with the administration of the compound systemically or locally to a tissue for the purpose of preventing some form of tissue degeneration or for restoring the degenerated tissue to its normal healthy level.

Other advantages of the invention include determining the tissue or tissues of origin of a given morphogen in order to administer a compound aimed at modulating the systemic level of morphogen for treatment of a disease or condition in which the level of morphogen production has become altered.

Brief Description of the Drawings

Fig. 1 shows the fragments of OP-1, used as probes in Northern hybridizations useful in the processes of the invention.

Fig. 2 shows results of Northern blot analysis of RNA using different OP-1-specific probes.

Fig. 3 shows results of Northern blot analysis of RNA from different cells types probed with an OP-1, probe.

Detailed Description

The invention is based on the discovery of a family of structurally related morphogenic proteins (BMPs), also called osteogenic proteins (OPs), and more particularly that various of these proteins play an important role, not only in embryogenesis, but also in tissue and organ maintenance and repair in juvenile and adult mammals. Morphogenic proteins which have been identified include BMP 2, 3, 4, 5, 6, OP-1 and OP-2 (murine and human), Vgr-1, Vgl, DPP, GDF-1, CMBP-2A, CMBP-2B, 60A, and the inhibin/activin class of proteins. Other recombinant proteins include COP1, COP3, COP4, COP5, COP7, and COP16. While, as explained herein, the morphogens have significant homologies and similarities in structure, it is hypothesized that variants within the morphogenic protein genes may have specific roles in specific tissue involving, for example, stimulation of progenitor cell multiplication, tissue specific or tissue preferred phenotype maintenance, and/or stimulation or modulation of the rate of differentiation, growth or replication of tissue cells characterized by high turnover. The effect on the long-term physiology, maintenance and repair of particular tissues by particular species of the morphogens is currently unknown in any significant detail. However, methods useful in determining which particular tissues express which particular morphogen(s), and for finding changes which stimulate or depress morphogen expression in vivo, would enable discovery and development of strategies for therapeutic treatment of a large number of diseased states, and provide drugs designed to implement the strategy.

This invention provides such methods and, more specifically, two generic processes for obtaining data which ultimately will permit determination of structure/activity relationships of specific naturally occurring mammalian morphogens and drugs capable of modulating their production. For example, using the assay of the invention, it has been determined that OP-1, first found in bone and demonstrated to be osteoinductive, is synthesized primarily in kidney, bladder, and adrenal tissue. This surprising discovery, coupled with the observation that patients with kidney disease often express loss of bone mass, suggests that the bone loss in these patients may be due to pathologic depression of OP-1 synthesis in kidney, and suggests that administration of OP-1 systemically or stimulation of OP-1 expression and secretion by the kidney may arrest bone loss, or effect remineralization through increased bone formation (i.e., osteogenesis).

There are two fundamental aspects of the invention. One aspect involves an assay to determine tissues and cell types capable of synthesis and secretion of the morphogens; the other involves the use of the identified cell types configured in a screening system to find substances useful therapeutically to modulate, i.e., stimulate or depress, morphogen expression and/or secretion.

The assay to determine the tissue of origin of a given morphogen involves screening a plurality (i.e., two or more) different tissues by determining a parameter indicative of production of a morphogen in the tissue, and comparing the parameters. The tissue(s) of origin will, of course, be the tissue that produces that morphogen.

The other assay of the invention involves screening candidate compounds for their ability to modulate the effective systemic or local concentration of a morphogen by incubating the compound with a cell culture that produces the morphogen, and assaying the culture for a parameter indicative of a change in the production level of the morphogen. Useful candidate compounds then may be tested for in vivo efficacy in a suitable animal model. These compounds then may be used in vivo to modulate effective morphogen concentration in the disease treatment.

1. Morphogen Tissue Distribution

Morphogens are broadly distributed in developing and adult tissue. For example, DPP and 60A are expressed in both embryonic and developing *Drosophila* tissue. Vgl has been identified in *Xenopus* embryonic tissue. Vgr-1 transcripts have been identified in a variety of murine tissues, including embryonic and developing brain, lung, liver, kidney and calvaria (dermal bone) tissue. In addition, both CBMP2B and CBMP3 have been identified in lung tissue. Recently, Vgr-1 transcripts also have been identified in adult murine lung, kidney, heart, and brain tissue, with particularly high levels in the lung (see *infra*). GDF-1 has been identified in human adult cerebellum and in fetal brain tissue. In addition, recent Northern blot analyses indicate that OP-1 is encoded by multiple transcripts in different tissues. This potential alternative splicing is consistent with the hypothesis that the longer transcripts may encode additional proteins (e.g., bicistronic mRNA) and each form may be tissue or developmentally related.

OP-1 and the CBMP2 proteins, both first identified as bone morphogens, have been identified in mouse and human placenta, hippocampus, calvaria and osteosarcoma tissue as determined by identification of OP-1 and CBMP2-specific sequences in cDNA libraries constructed from these tissues (see USSN 422,699, incorporated herein by reference). Additionally, the OP-1 protein is present in a variety of embryonic and developing tissues including kidney, liver, heart and brain as determined by Western blot analysis and immunolocalization (see *infra*). OP-1-specific transcripts also have been identified in both embryonic and developing tissues, most abundantly in developing kidney, bladder, adrenal and (see *infra*). OP-1 also has been identified as a mesoderm inducing factor present during embryogenesis. Moreover, OP-1 has been shown to be associated with satellite cells in the muscle and associated with potential pluripotential stem cells in bone marrow following damage to adult murine endochondral bone, indicating its morphogenic role in tissue repair and regeneration. In addition, a novel protein GDF-1 comprising a 7 cysteine skeleton, has been identified in neural tissue (Lee, 1991, *Proc. Nat. Aca. Sci.* 88: 4250-4254).

Knowledge of the tissue distribution of a given morphogen may be useful in choosing a cell type for screening according to the invention, or for targeting that cell type or tissue type for treatment. The proteins (or their mRNA transcripts) are readily identified in different tissues using standard methodologies and minor modifications thereof in tissues where expression may be low. For example, protein distribution may be determined using standard Western blot analysis or immunocytochemical techniques, and antibodies specific to the morphogen or

morphogens of interest. Similarly, the distribution of morphogen transcripts may be determined using standard Northern hybridization protocols and a transcript-specific probe and hybridization conditions.

2. Useful Morphogens

As defined herein a protein is morphogenic if it is capable of inducing the developmental cascade of cellular and molecular events that culminate in the formation of new, organ-specific tissue and comprises at least the conserved C-terminal six cysteine skeleton or its functional equivalent (see supra). Specifically, the morphogens generally are capable of all of the following biological functions in a morphogenically permissive environment: stimulating proliferation of progenitor cells; stimulating the differentiation of progenitor cells; stimulating the proliferation of differentiated cells; and supporting the growth and maintenance of differentiated cells, including the "redifferentiation" of transformed cells. Details of how the morphogens detectable according to the methods of this invention first were identified, as well as a description on how to make, use and test them for morphogenic activity are disclosed in USSN 667,274, filed March 11, 1991 and USSN 752,764, filed August 30, 1991, the disclosures of which are hereby incorporated by reference. As disclosed therein, the morphogens may be purified from naturally-sourced material or recombinantly produced from procaryotic or eucaryotic host cells, using the genetic sequences disclosed therein. Alternatively, novel morphogenic sequences may be identified following the procedures disclosed therein.

Particularly useful proteins include those which comprise the naturally derived sequences disclosed in Table II. Other useful sequences include biosynthetic constructs such as those disclosed in U.S. Pat. 5,011,691, the disclosure of which is incorporated herein by reference (e.g., COP-1, COP-3, COP-4, COP-5, COP-7, and COP-16).

Accordingly, the morphogens detectable according to the methods and compositions of this invention also may be described by morphogenically active proteins having amino acid sequences sharing 70% or, preferably, 80% homology (similarity) with any of the sequences described above, where "homology" is as defined herein above.

The morphogens detectable according to the method of this invention also can be described by any of the 6 generic sequences described herein (Generic Sequences 1, 2, 3, 4, 5 and 6). Generic sequences 1 and 2 also may include, at their N-terminus, the sequence

Cys Xaa Xaa Xaa Xaa (Seq. ID No. 15)

1

5

Table II, set forth below, compares the amino acid sequences of the active regions of native proteins that have been identified as morphogens, including human OP-1 (hOP-1, Seq. ID Nos. 5 and 16-17), mouse OP-1 (mOP-1, Seq. ID Nos. 6 and 18-19), human and mouse OP-2 (Seq. ID Nos. 7, 8, and 20-23), CBMP2A (Seq. ID No. 9), CBMP2B (Seq. ID No. 10), BMP3 (Seq. ID No. 26), DPP (from Drosophila, Seq. ID No. 11), Vgl, (from Xenopus, Seq. ID No. 12), Vgr-1 (from mouse, Seq. ID No. 13), GDF-1 (from mouse, Seq. ID

Nos. 14, 32 and 33), 60A protein (from *Drosophila*, Seq. ID Nos. 24 and 25), BMP5 (Seq. ID No. 27) and BMP6 (Seq. ID No. 28). The sequences are aligned essentially following the method of Needleman et al. (1970) J. Mol. Biol., 48:443-453, calculated using the Align Program (DNASTAR, Inc.) In the table, three dots indicates that the amino acid in that position is the same as the amino acid in hOP-1. Three dashes indicates that no amino acid is present in that position, and are included for purposes of illustrating homologies. For example, amino acid residue 60 of CBMP-2A and CBMP-2B is "missing". Of course, both these amino acid sequences in this region comprise Asn-Ser (residues 58, 59), with CBMP-2A then comprising Lys and Ile, whereas CBMP-2B comprises Ser and Ile.

TABLE II

| | | | | | | | | |
|---------|-----|-----|-----|-----|-----|-----|-----|-----|
| hOP-1 | Cys | Lys | Lys | His | Glu | Leu | Tyr | Val |
| mOP-1 | ... | ... | ... | ... | ... | ... | ... | ... |
| hOP-2 | ... | Arg | Arg | ... | ... | ... | ... | ... |
| mOP-2 | ... | Arg | Arg | ... | ... | ... | ... | ... |
| DPP | ... | Arg | Arg | ... | Ser | ... | ... | ... |
| Vgl | ... | ... | Lys | Arg | His | ... | ... | ... |
| Vgr-1 | ... | ... | ... | ... | Gly | ... | ... | ... |
| CBMP-2A | ... | ... | Arg | ... | Pro | ... | ... | ... |
| CBMP-2B | ... | Arg | Arg | ... | Ser | ... | ... | ... |
| BMP3 | ... | Ala | Arg | Arg | Tyr | ... | Lys | ... |
| GDF-1 | ... | Arg | Ala | Arg | Arg | ... | ... | ... |
| 60A | ... | Gln | Met | Glu | Thr | ... | ... | ... |
| BMP5 | ... | ... | ... | ... | ... | ... | ... | ... |
| BMP6 | ... | Arg | ... | ... | ... | ... | ... | ... |
| | 1 | | | | 5 | | | |

| | | | | | | | | | |
|---------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| hOP-1 | Ser | Phe | Arg | Asp | Leu | Gly | Trp | Gln | Asp |
| mOP-1 | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| hOP-2 | ... | ... | Gln | ... | ... | ... | ... | Leu | ... |
| mOP-2 | Ser | ... | ... | ... | ... | ... | ... | Leu | ... |
| DPP | Asp | ... | Ser | ... | Val | ... | ... | Asp | ... |
| Vgl | Glu | ... | Lys | ... | Val | ... | ... | ... | Asn |
| Vgr-1 | ... | ... | Gln | ... | Val | ... | ... | ... | ... |
| CBMP-2A | Asp | ... | Ser | ... | Val | ... | ... | Asn | ... |
| CBMP-2B | Asp | ... | Ser | ... | Val | ... | ... | Asn | ... |
| BMP3 | Asp | ... | Ala | ... | Ile | ... | ... | Ser | Glu |
| GDF-1 | ... | ... | ... | Glu | Val | ... | ... | His | Arg |
| 60A | Asp | ... | Lys | ... | ... | ... | ... | His | ... |

| | | | | | | | | | |
|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| BMP5 | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| BMP6 | ... | ... | Gln | ... | ... | ... | ... | ... | ... |
| | | 10 | | | | | 15 | | |

| | | | | | | | | | |
|---------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| hOP-1 | Trp | Ile | Ile | Ala | Pro | Glu | Gly | Tyr | Ala |
| mOP-1 | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| hOP-2 | ... | Val | ... | ... | ... | Gln | ... | ... | Ser |
| mOP-2 | ... | Val | ... | ... | ... | Gln | ... | ... | Ser |
| DPP | ... | ... | Val | ... | ... | Leu | ... | ... | Asp |
| Vgl | ... | Val | ... | ... | ... | Gln | ... | ... | Met |
| Vgr-1 | ... | ... | ... | ... | ... | Lys | ... | ... | ... |
| CBMP-2A | ... | ... | Val | ... | ... | Pro | ... | ... | His |
| CBMP-2B | ... | ... | Val | ... | ... | Pro | ... | ... | Gln |
| BMP3 | ... | ... | ... | Ser | ... | Lys | Ser | Phe | Asp |
| GDF-1 | ... | Val | ... | ... | ... | Arg | ... | Phe | Leu |
| 60A | ... | ... | ... | ... | ... | ... | ... | ... | Gly |
| BMP5 | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| BMP6 | ... | ... | ... | ... | ... | Lys | ... | ... | ... |
| | | | 20 | | | | | 25 | |

| | | | | | | | | | |
|---------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| hOP-1 | Ala | Tyr | Tyr | Cys | Glu | Gly | Glu | Cys | Ala |
| mOP-1 | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| hOP-2 | ... | ... | ... | ... | ... | ... | ... | ... | Ser |
| mOP-2 | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| DPP | ... | ... | ... | ... | His | ... | Lys | ... | Pro |
| Vgl | ... | Asn | ... | ... | Tyr | ... | ... | ... | Pro |
| Vgr-1 | ... | Asn | ... | ... | Asp | ... | ... | ... | Ser |
| CBMP-2A | ... | Phe | ... | ... | His | ... | Glu | ... | Pro |
| CBMP-2B | ... | Phe | ... | ... | His | ... | Asp | ... | Pro |
| BMP3 | ... | ... | ... | ... | Ser | ... | Ala | ... | Gln |
| GDF-1 | ... | Asn | ... | ... | Gln | ... | Gln | ... | ... |
| 60A | ... | Phe | ... | ... | Ser | ... | ... | ... | Asn |
| BMP5 | ... | Phe | ... | ... | Asp | ... | ... | ... | Ser |
| BMP6 | ... | Asn | ... | ... | Asp | ... | ... | ... | Ser |
| | | | | 30 | | | | | 35 |

46

| | | | | | | | | | |
|---------|-----|-----|-----|-----|-----|-----|-----|-------|-----|
| hOP-1 | Phe | Pro | Leu | Asn | Ser | Tyr | Met | Asn | Ala |
| mOP-1 | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| hOP-2 | ... | ... | ... | Asp | ... | Cys | ... | ... | ... |
| mOP-2 | ... | ... | ... | Asp | ... | Cys | ... | ... | ... |
| DPP | ... | ... | ... | Ala | Asp | His | Phe | ... | Ser |
| Vgl | Tyr | ... | ... | Thr | Glu | Ile | Leu | ... | Gly |
| Vgr-1 | ... | ... | ... | ... | Ala | His | ... | ... | ... |
| CBMP-2A | ... | ... | ... | Ala | Asp | His | Leu | ... | Ser |
| CBMP-2B | ... | ... | ... | Ala | Asp | His | Leu | ... | Ser |
| GDF-1 | Leu | ... | Val | Ala | Leu | Ser | Gly | Ser** | ... |
| BMP3 | ... | ... | Met | Pro | Lys | Ser | Leu | Lys | Pro |
| 60A | ... | ... | ... | ... | Ala | His | ... | ... | ... |
| BMP5 | ... | ... | ... | ... | Ala | His | Met | ... | ... |
| BMP6 | ... | ... | ... | ... | Ala | His | Met | ... | ... |

40

| | | | | | | | | | |
|---------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| hOP-1 | Thr | Asn | His | Ala | Ile | Val | Gln | Thr | Leu |
| mOP-1 | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| hOP-2 | ... | ... | ... | ... | ... | Leu | ... | Ser | ... |
| mOP-2 | ... | ... | ... | ... | ... | Leu | ... | Ser | ... |
| DPP | ... | ... | ... | ... | Val | ... | ... | ... | ... |
| Vgl | Ser | ... | ... | ... | ... | Leu | ... | ... | ... |
| Vgr-1 | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| CBMP-2A | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| CBMP-2B | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| BMP3 | Ser | ... | ... | ... | Thr | Ile | ... | Ser | Ile |
| GDF-1 | Leu | ... | ... | ... | Val | Leu | Arg | Ala | ... |
| 60A | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| BMP5 | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| BMP6 | ... | ... | ... | ... | ... | ... | ... | ... | ... |

45

50

| | | | | | | | | | |
|---------|-----|-----|-------|-----|-----|-----|-----|-----|-----|
| hOP-1 | Val | His | Phe | Ile | Asn | Pro | Glu | Thr | Val |
| mOP-1 | ... | ... | ... | ... | ... | ... | Asp | ... | ... |
| hOP-2 | ... | His | Leu | Met | Lys | ... | Asn | Ala | ... |
| mOP-2 | ... | His | Leu | Met | Lys | ... | Asp | Val | ... |
| DPP | ... | Asn | Asn | Asn | ... | ... | Gly | Lys | ... |
| Vgl | ... | ... | Ser | ... | Glu | ... | ... | Asp | Ile |
| Vgr-1 | ... | ... | Val | Met | ... | ... | ... | Tyr | ... |
| CBMP-2A | ... | Asn | Ser | Val | ... | Ser | --- | Lys | Ile |
| CBMP-2B | ... | Asn | Ser | Val | ... | Ser | --- | Ser | Ile |
| BMP3 | ... | Arg | Ala** | Gly | Val | Val | Pro | Gly | Ile |
| GDF-1 | Met | ... | Ala | Ala | Ala | ... | Gly | Ala | Ala |
| 60A | ... | ... | Leu | Leu | Glu | ... | Lys | Lys | ... |
| BMP5 | ... | ... | Leu | Met | Phe | ... | Asp | His | ... |
| BMP6 | ... | ... | Leu | Met | ... | ... | ... | Tyr | ... |
| | | 55 | | | | | 60 | | |

| | | | | | | | | | |
|---------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| hOP-1 | Pro | Lys | Pro | Cys | Cys | Ala | Pro | Thr | Gln |
| mOP-1 | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| hOP-2 | ... | ... | Ala | ... | ... | ... | ... | ... | Lys |
| mOP-2 | ... | ... | Ala | ... | ... | ... | ... | ... | Lys |
| DPP | ... | ... | Ala | ... | ... | Val | ... | ... | ... |
| Vgl | ... | Leu | ... | ... | ... | Val | ... | ... | Lys |
| Vgr-1 | ... | ... | ... | ... | ... | ... | ... | ... | Lys |
| CBMP-2A | ... | ... | Ala | ... | ... | Val | ... | ... | Glu |
| CBMP-2B | ... | ... | Ala | ... | ... | Val | ... | ... | Glu |
| BMP3 | ... | Glu | ... | ... | ... | Val | ... | Glu | Lys |
| GDF-1 | Asp | Leu | ... | ... | ... | Val | ... | Ala | Arg |
| 60A | ... | ... | ... | ... | ... | ... | ... | ... | Arg |
| BMP5 | ... | ... | ... | ... | ... | ... | ... | ... | Lys |
| BMP6 | ... | ... | ... | ... | ... | ... | ... | ... | Lys |
| | | | 65 | | | | | 70 | |

| | | | | | | | | | |
|---------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| hOP-1 | Leu | Asn | Ala | Ile | Ser | Val | Leu | Tyr | Ph |
| mOP-1 | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| hOP-2 | ... | Ser | ... | Thr | ... | ... | ... | ... | Tyr |
| mOP-2 | ... | Ser | ... | Thr | ... | ... | ... | ... | Tyr |
| Vgl | Met | Ser | Pro | ... | ... | Met | ... | Phe | Tyr |
| Vgr-1 | Val | ... | ... | ... | ... | ... | ... | ... | ... |
| DPP | ... | Asp | Ser | Val | Ala | Met | ... | ... | Leu |
| CBMP-2A | ... | Ser | ... | ... | ... | Met | ... | ... | Leu |
| CBMP-2B | ... | Ser | ... | ... | ... | Met | ... | ... | Leu |
| BMP3 | Met | Ser | Ser | Leu | ... | Ile | ... | Phe | Tyr |
| GDF-1 | ... | Ser | Pro | ... | ... | ... | ... | Phe | ... |
| 60A | ... | Gly | ... | Leu | Pro | ... | ... | ... | His |
| BMP5 | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| BMP6 | ... | ... | ... | ... | ... | ... | ... | ... | ... |

75

80

| | | | | | | | | | |
|---------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| hOP-1 | Asp | Asp | Ser | Ser | Asn | Val | Ile | Leu | Lys |
| mOP-1 | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| hOP-2 | ... | Ser | ... | Asn | ... | ... | ... | ... | Arg |
| mOP-2 | ... | Ser | ... | Asn | ... | ... | ... | ... | Arg |
| DPP | Asn | ... | Gln | ... | Thr | ... | Val | ... | ... |
| Vgl | ... | Asn | Asn | Asp | ... | ... | Val | ... | Arg |
| Vgr-1 | ... | ... | Asn | ... | ... | ... | ... | ... | ... |
| CBMP-2A | ... | Glu | Asn | Glu | Lys | ... | Val | ... | ... |
| CBMP-2B | ... | Glu | Tyr | Asp | Lys | ... | Val | ... | ... |
| BMP3 | ... | Glu | Asn | Lys | ... | ... | Val | ... | ... |
| GDF-1 | ... | Asn | ... | Asp | ... | ... | Val | ... | Arg |
| 60A | Leu | Asn | Asp | Glu | ... | ... | Asn | ... | ... |
| BMP5 | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| BMP6 | ... | ... | Asn | ... | ... | ... | ... | ... | ... |

85

| | | | | | | | | |
|---------|-----|-----|-----|-----|-----|-----|-----|-----|
| hOP-1 | Lys | Tyr | Arg | Asn | Met | Val | Val | Arg |
| mOP-1 | ... | ... | ... | ... | ... | ... | ... | ... |
| hOP-2 | ... | His | ... | ... | ... | ... | ... | Lys |
| mOP-2 | ... | His | ... | ... | ... | ... | ... | Lys |
| DPP | Asn | ... | Gln | Glu | ... | Thr | ... | Val |
| Vgl | His | ... | Glu | ... | ... | Ala | ... | Asp |
| Vgr-1 | ... | ... | ... | ... | ... | ... | ... | ... |
| CBMP-2A | Asn | ... | Gln | Asp | ... | ... | ... | Glu |
| CBMP-2B | Asn | ... | Gln | Glu | ... | ... | ... | Glu |
| BMP3 | Val | ... | Pro | ... | ... | Thr | ... | Glu |
| GDF-1 | Gln | ... | Glu | Asp | ... | ... | ... | Asp |
| 60A | ... | ... | ... | ... | ... | Ile | ... | Lys |
| BMP5 | ... | ... | ... | ... | ... | ... | ... | ... |
| BMP6 | ... | ... | ... | Trp | ... | ... | ... | ... |
| | 90 | | | | | 95 | | |

| | | | | | |
|---------|-----|-----|-----|-----|-----|
| hOP-1 | Ala | Cys | Gly | Cys | His |
| mOP-1 | ... | ... | ... | ... | ... |
| hOP-2 | ... | ... | ... | ... | ... |
| mOP-2 | ... | ... | ... | ... | ... |
| DPP | Gly | ... | ... | ... | Arg |
| Vgl | Glu | ... | ... | ... | Arg |
| Vgr-1 | ... | ... | ... | ... | ... |
| CBMP-2A | Gly | ... | ... | ... | Arg |
| CBMP-2B | Gly | ... | ... | ... | Arg |
| BMP3 | Ser | ... | Ala | ... | Arg |
| GDF-1 | Glu | ... | ... | ... | Arg |
| 60A | Ser | ... | ... | ... | ... |
| BMP5 | Ser | ... | ... | ... | ... |
| BMP6 | ... | ... | ... | ... | ... |

****Between residues 56 and 57 of BMP3 is a Val residue ;
between residues 43 and 44 of GDF-1 lies
the amino acid sequence Gly-Gly-Pro-Pro.**

As is apparent from the foregoing amino acid sequence comparisons, significant amino acid changes can be made within the generic sequences while retaining the morphogenic activity. For example, while the GDF-1 protein sequence depicted in Table II shares only about 50% amino acid identity with the hOP1 sequence described therein, the GDF-1 sequence shares greater than 70% amino acid sequence homology (or "similarity") with the hOP1 sequence, where "homology" or "similarity" includes allowed conservative amino acid changes within the sequence as defined by Dayoff, et al., Atlas of Protein Sequence and Structure vol.5, supp.3, pp.345-362, (M.O. Dayoff, ed., Nat'l BioMed. Res. Fd'n, Washington D.C. 1979.)

The currently most preferred protein sequences detectable as morphogens in this invention include those having greater than 60% identity, preferably greater than 65% identity, with the amino acid sequence defining the conserved six cysteine skeleton of hOP1 (e.g., residues 43-139 of Seq. ID No. 5). These most preferred sequences include both allelic and species variants of the OP-1 and OP-2 proteins, including the *Drosophila* 60A protein. Accordingly, in still another preferred aspect, the invention includes detection of morphogens comprising species of polypeptide chains having the generic amino acid sequence referred to herein as "OPX", which defines the seven cysteine skeleton and accommodates the identities between the various identified mouse and human OP1 and OP2 proteins. OPX is presented in Seq. ID No. 29. As described therein, each Xaa at a given position

ind p ndently is sel cted from the residues ccurring at the corresp nding position in the C-terminal sequence of mouse or human OP1 or OP2 (see Seq. ID Nos. 5-8 and/or Seq. ID Nos. 16-23).

3. Tissue-Specific Expression of OP-1

Once a morphogen is identified in a tissue, its level may be determined either at the protein or nucleic acid level. By comparing the levels of production of a given morphogen among different tissues, it is possible to determine the tissue(s) of origin of that morphogen. The level of production of the morphogen OP-1 in different tissues is one example of a morphogen having a tissue of origin, i.e., the kidney, which contains a cell type that can also be used as the cell type which is used to screen, according to the invention, different compounds for their potential effects on morphogen (OP-1) production.

The level of OP-1 varies among different tissue types. In order to screen compounds for their effect on the production of OP-1 by a given cell type, it may be desirable to determine which tissues produce levels of OP-1 which are sufficiently high to show a potential decrease and sufficiently low to show a potential increase in production. Different tissues may be screened at the RNA level as follows.

Any probe capable of hybridizing specifically to a transcript, and distinguishing the transcript of interest from other, related transcripts may be used. Because the morphogens to be detected in the methods of this invention share such high sequence homology in their C-terminal domain, the tissue distribution of a specific morphogen transcript may best be determined using a probe specific

for the "pro" region of the immature protein and/or the N-terminal heterologous region of the mature protein. Another useful probe sequence is the 3' non-coding region immediately following the stop codon. These portions of the sequence vary substantially among the morphogens of this invention, and accordingly, are specific for each protein. For example, a particularly useful Vgr-1-specific probe sequence is the PvuII-SacI fragment, a 265-bp fragment encoding both a portion of the pro region and the N-terminus of the mature sequence. Similarly, particularly useful mOP-1-specific probe sequences are the BstXI-BglI fragment, a 0.68kb sequence that covers approximately two-thirds of the mOP1 pro region; a StuI-StuI fragment, a 0.2 kb sequence immediately upstream of the 7-cysteine domain, and an EcoRI-PstI fragment, a 0.3kb fragment containing the 3'untranslated sequence. Similar approaches may be used, for example, with hOP-1 (SEQ. ID NO.16) or human or mouse OP-2 (SEQ. ID NOS.20 and 22).

Using morphogen-specific oligonucleotide probes, morphogen transcripts can be identified in mammalian tissues, using standard methodologies well known to those having ordinary skill in the art. Briefly, total RNA from mouse embryos and organs from post-natal animals is prepared using the acid guanidine thiocyanate-phenol-chloroform method (Chomczynski et al., Anal. Biochem. 162:156-159, 1987). The RNA may be dissolved in TES buffer (10 mM Tris-HCl, 1 mM EDTA, 0.1% SDS, pH 7.5) and treated with Proteinase K (approx. 1.5 mg per g tissue sample) at 45°C for 1 hr. Poly(A)⁺ RNA selection on oligo(dT)-cellulose (Type 7, Pharmacia LKB Biotechnology Inc., Piscataway, NJ) may be done in a batch procedure by mixing 0.1 g oligo(dT)-cellulose with 11 ml RNA solution (from 1 g

tissue) in TES buffer and 0.5 M NaCl). Then after the oligo(dT) cellulose is washed in binding buffer (0.5 M NaCl, 10 mM Tris-HCl, 1 mM EDTA, pH 7.5) and poly(A)⁺ RNA is eluted with water. Poly(A)⁺ RNA (5 or 15 µg/lane) is fractionated on 1 or 1.2% agarose-formaldehyde gels (Selden, in Current Protocols in Molecular Biology, Ausubel et al. eds., pp. 1-4, 8, 9, Greene Publishing and Wiley-Interscience, New York, 1991). 1 µl of 400 µg/ml ethidium bromide is added to each sample prior to heat denaturation (Rosen et al., Focus 12:23-24, 1990). Following electrophoresis, the gels are photographed and the RNA is blotted overnight onto Nytran nitrocellulose membranes (Schleicher & Schuell Inc., Keene, NH) with 10 x SSC. The membranes are baked at 80°C for 30-60 min. and irradiated with UV light (1 mW/cm² for 25 sec.). The Northern hybridization conditions may be as previously described (Ozkaynak et al., EMBO J. 9:2085-2093, 1990). For re-use, the filters may be deprobed in 1 mM Tris-HCl, 1 mM EDTA, 0.1% SDS, pH 7.5, at 90-95°C and exposed to film to assure complete removal of previous hybridization signals.

One probe which may be used to screen for transcripts encoding a morphogen includes a portion of or the complete OP-1 cDNA, which may be used to detect the presence of OP-1 mRNA or mRNAs of related morphogens. The sequence of the murine cDNA gene is set forth in SEQ ID NO:14.

OP-1 mRNA expression was analyzed in 17 day mouse embryos and 3 day post-natal mice by sequentially hybridizing filters with various probes. Probes from regions other than the highly conserved 7-cysteine domain were selected because this region is highly variable among

members of the TGF- β superfamily. Fig. 1 shows the fragments of OP-1, used as probes in the Northern hybridizations. The solid box indicates the putative signal peptide and the hatched box corresponds to the TGF- β -like domain that contains the seven cysteine residues. Asterisks indicate the potential N-glycosylation sites. The arrow marks the location of the cleavage site for OP-1 maturation. Three solid bars below the diagram indicate the OP-1 specific fragments used in making ^{32}P -labeled probes (0.68 kb BstXI - BglI fragment, 0.20 kb StuI - StuI fragment and 0.34 kb EarI - PstI non-coding fragment).

Hybridization with a probe that covers approximately two thirds of the pro region (the 0.68 kb BstXI-BglI fragment), reveals a 4 kb message and 3 messages at 1.8 kb, 2.2 kb and 2.4 kb (Fig. 2B and D, and Fig. 3). In the Northern hybridization of Fig. 2, equal amounts (15 μg) of poly(A)⁺ RNA were loaded into each lane, electrophoresed on a 1% agarose-formaldehyde gel, blotted and hybridized. A 0.24 - 9.49 kb RNA ladder (Bethesda Research Labs, Inc.) was used as size standard. The same filter was used for sequential hybridizations with labeled probes specific for OP-1 (Panels B and D), Vgr-1 (Panel C), and EF-Tu (Panel A). Panel A: the EF-Tu specific probe (a control) was the 0.4 kb HindIII-SacI fragment (part of the coding region), the SacI site used belonged to the vector; Panel B: the OP-1 specific probe was the 0.68 kb BstXI-BglI fragment (two thirds of the pro region and upstream sequences of the mature domain, not including any sequences from the 7-cysteine domain); Panel C: the Vgr-1 specific probe was the 0.26 kb PvuII-SacI fragment (part of the pro region and the amino-terminal sequences of the mature

domain, including the first cysteine) (Ly ns t al., 1989, Proc. Nat. Aca. Sci. 86: 4554, hereby incorporated by reference). Panel D: the OP-1 (3' flanking) specific probe was the 0.34 kb *E*arI-*P*stI fragment (3' untranslated sequences immediately following the sequences encoding OP-1).

In Fig. 3, the tissues to be used for RNA preparation were obtained from two week old mice (Panel A) or 5 week old mice (Panel B), with the exception of poly A+ RNA which was obtained from kidney adrenal gland of two week old mice (Panel B). Equal amounts of poly A+ RNA (15 μ g for Panel A and 5 μ g for Panel B) were loaded into each well. After electrophoresis (1.2% agarose-formaldehyde gels) and blotting, RNA was hybridized to the OP-1 specific 3' flanking probe described in the legend of Fig. 2 (Panel D). The 0.24-9.5 kb RNA ladder was used as size standard. The arrowheads indicate the OP-1 specific messages. The lower section of Panels A and B show the hybridization pattern obtained with the EF-Tu specific probe (a control).

Although the size of the Vgr-1 specific message is close to the 4 kb OP-1 species (Fig. 2 Panel C), the OP-1 4 kb mRNA is somewhat larger. To further rule out cross-hybridization with a non-OP-1 message, the 0.2 kb *S*tuI-*S*tuI fragment which represents the gene specific sequences immediately upstream of those encoding the 7-cysteine domain was used. This probe gave a hybridization pattern similar to the one shown in Fig. 2 Panel B (data not shown). A third probe, the 0.34 kb *E*arI-*P*stI fragment containing 3' untranslated sequences, also confirmed the pattern (Fig. 2 Panel D). Thus, the same four OP-1 specific messages were observed with three distinct probes.

The appearance of a new 4 kb OP-1 mRNA species was initially interpreted as cross hybridization of the OP-1 probe with Vgr-1 mRNA, which is approximately this size (Fig. 2 Panel C). However, the 4 kb message was detected with three different OP-1 specific probes, including one specific to the 3' untranslated region, and moreover it was separated from Vgr-1 message on the basis of size. Most likely, therefore, the 4 kb mRNA (and the three species of 1.8 kb, 2.2 kb and 2.4 kb) results from alternative splicing of OP-1 transcripts. The 4 kb OP-1 mRNA could also represent a bicistronic mRNA. The 4 kb message is a minor species in kidney, while it is more prominent in adrenal tissue.

The level of OP-1 expression was compared in different tissues using poly(A)⁺ RNA prepared from brain, spleen, lung, kidney and adrenal gland, heart, and liver of 13 day post-natal mice. The RNA was analyzed on Northern blots by hybridization to various probes (Fig. 3. Equal amounts of mRNA, as judged by optical density, were fractionated on agarose formaldehyde gels. Ethidium bromide staining of the gels revealed some residual ribosomal RNA in addition to the mRNA and provided another assurance that the mRNA was not degraded and that there was not significant quantitative or qualitative variation in the preparation. As control for mRNA recovery, EF-Tu (translational elongation factor) mRNA was probed (assuming uniform expression of EF-Tu in most tissues). A great variation in the level of OP-1 expression was observed in spleen, lung, kidney and adrenal tissues whereas EF-Tu mRNA levels appeared relatively constant in these tissues (Fig. 3 Panel A). The highest level of OP-1 mRNA was found in the kidneys. Uniformly lower levels of EF-Tu mRNA were

found in brain, heart and liver (Fig. 3 Panel A). Additional analysis of OP-1 mRNA showed the presence of significant amounts of OP-1 mRNA in the bladder (data not shown). In summary, next to kidney, bladder and adrenal tissue, brain tissue contained the highest levels of OP-1 RNA, whereas heart and liver did not give detectable signals.

OP-1 mRNA patterns display qualitative changes in the various tissues. Of the four messages found in brain, the 2.2 kb message is most abundant whereas in lung and spleen the 1.8 kb message predominates. Levels of the 1.8-2.4 kb in the kidney OP-1 mRNA are approximately two times higher in 3 day post-natal mice than in 17 day embryos, perhaps reflecting phases in bone and/or kidney development. mRNA was also prepared from carefully separated renal and adrenal tissues of 5 week old mice. Northern blot analysis (Figure 4, Panel B) revealed that the high levels of 2.2 kb mRNA were derived from renal tissue whereas the 4 kb mRNA was more prominent in adrenal tissue.

The detection of OP-1 message primarily in the kidney but also in bladder links OP-1 expression specifically with the urinary tract. Interestingly, the related Vgr-1 is also expressed at significant levels in kidney although its main site of expression in lung.

Once the tissue-specific expression of a given morphogen is known, cell types known to exist in that tissue or cell lines derived from that tissue can be screened, in a similar manner, to identify the cell type within that tissue that is actually responsible for the tissue specific synthesis and secretion of the morphogen. Once a cell type which produces the morphogen in an amount

sufficient to detect increases or decreases in the production level of the morphogen upon exposure to a compound is identified, it may be used in tissue culture assay to rapidly screen for the ability of compound to upregulate or down regulate the synthesis and secretion of the morphogen. The level of morphogen production by the chosen cell type is determined with and without incubating the cell in culture with the compound, in order to assess the effects of the compound on the cell's ability to synthesize or secrete the morphogen. This can be accomplished by detection of the level of production of the morphogen either at the protein or mRNA level.

4. Growth of Cells in Culture

Cell cultures derived from kidney, adrenals, urinary bladder, brain, or other organs, may be prepared as described widely in the literature. For example, kidneys may be explanted from neonatal, new born, young or adult rodents (mouse or rat) and used in organ culture as whole or sliced (1-4 mm) tissues. Primary tissue cultures and established cell lines, also derived from kidney, adrenals, urinary, bladder, brain, or other tissues may be established in multiwell plates (6 well, 24 well, or 96 well) according to conventional cell culture techniques, and are cultured in the absence or presence of serum for a period of time (1-7 days). Cells may be cultured, for example, in Dulbecco's Modified Eagle medium (Gibco, Long Island, NY) containing serum (e.g., fetal calf serum at 1%-10%, Gibco) or in serum-deprived medium, as desired, or in defined medium (e.g., containing insulin, transferrin, glucose, albumin, or other growth factors).

Samples for testing the level of morphogen production include culture supernatants or cell lysates, collected periodically and evaluated for OP-1 production by immunoblot analysis of a portion of the cell culture itself, collected periodically and used to prepare polyA+ RNA for RNA analysis (Sambrook et al., eds., Molecular Cloning, 1989, Cold Spring Harbor Press, Cold Spring Harbor, NY). To monitor de novo OP-1 synthesis, some cultures are labeled with ^{35}S -methionine/ ^{35}S -cysteine mixture for 6-24 hours and then evaluated for morphogen production by conventional immunoprecipitation methods (Sambrook et al., eds., Molecular Cloning, 1989, Cold Spring Harbor Press, Cold Spring Harbor, NY). Alternatively, the production of morphogen or determination of the level of morphogen production may be ascertained using a simple assay for a parameter of cell growth, e.g., cellular proliferation or death. For example, where a morphogen is produced by a cultured cell line, the addition of antibody specific for the morphogen may result in relief from morphogen inhibition of cell growth. Thus, measurement of cellular proliferation can be used as an indication of morphogen production by a tissue.

5. Determination of Level of Morphogenic Protein

In order to quantitate the production of a morphogenic protein by a cell type, an immunoassay may be performed to detect the morphogen using a polyclonal or monoclonal antibody specific for that morphogen. For example, OP-1 may be detected using a polyclonal antibody specific for OP-1 in an ELISA, as follows.

1 μg /100 μl of affinity-purified polyclonal rabbit IgG specific for OP-1 is added to each well of a 96-well

plate and incubated at 37°C for an hour. The wells are washed four times with 0.16M sodium borate buffer with 0.15 M NaCl (BSB), pH 8.2, containing 0.1% Tween 20. To minimize non-specific binding, the wells are blocked by filling completely with 1% bovine serum albumin (BSA) in BSB for 1 hour at 37°C. The wells are then washed four times with BSB containing 0.1% Tween 20. A 100 ul aliquot of an appropriate dilution of each of the test samples of cell culture supernatant is added to each well in triplicate and incubated at 37°C for 30 min. After incubation, 100 ul biotinylated rabbit anti-OP-1 serum (stock solution is about 1 mg/ml and diluted 1:400 in BSB containing 1% BSA before use) is added to each well and incubated at 37°C for 30 min. The wells are then washed four times with BSB containing 0.1% Tween 20. 100 ul streptavidin-alkaline (Southern Biotechnology Associates, Inc. Birmingham, Alabama, diluted 1:2000 in BSB containing 0.1% Tween 20 before use) is added to each well and incubated at 37°C for 30 min. The plates are washed four times with 0.5M Tris buffered Saline (TBS), pH 7.2. 50ul substrate (ELISA Amplification System Kit, Life Technologies, Inc., Bethesda, MD) are added to each well incubated at room temperature for 15 min. Then, 50 ul amplifier (from the same amplification system kit) is added and incubated for another 15 min at room temperature. The reaction is stopped by the addition of 50 ul 0.3 M sulphuric acid. The OD at 490 nm of the solution in each well is recorded. To quantitate OP-1 in culture media, a OP-1 standard curve is performed in parallel with the test samples.

6. Preparation of Polyclonal Antibody

Polyclonal antibody is prepared as follows. Each rabbit is given a primary immunization of 100 ug/500 ul E. coli-produced OP-1 monomer (amino acids 328-431 of SEQ. ID NO: 11) in 0.1% SDS mixed with 500 ul Complete Freund's Adjuvant. The antigen is injected subcutaneously at multiple sites on the back and flanks of the animal. The rabbit is boosted after a month in the same manner using incomplete Freund's Adjuvant. Test bleeds are taken from the ear vein seven days later. Two additional boosts and test bleeds are performed at monthly intervals until antibody against OP-1 is detected in the serum using an ELISA assay. Then, the rabbit is boosted monthly with 100 ug of antigen and bled (15 ml per bleed) at days seven and ten after boosting.

7. Preparation of Monoclonal Antibody and Neutralizing Monoclonal Antibody

Monoclonal antibody specific for a given morphogen may be prepared as follows. A mouse is given two injections of E. coli produced OP-1 monomer (amino acids 328-431 in SEQ ID NO:11). The first injection contains 100ug of OP-1 in complete Freund's adjuvant and is given subcutaneously. The second injection contains 50 ug of OP-1 in incomplete adjuvant and is given intraperitoneally. The mouse then receives a total of 230 ug of OP-1 (amino acids 307-431 of SEQ ID NO:11) in four intraperitoneal injections at various times over an eight month period. One week prior to fusion, The mouse is boosted intraperitoneally with 100 ug of OP-1 (15-139) and 30 ug of the N-terminal peptide (Ser293-Asn309-Cys) conjugated through the added cys residue to bovine serum albumin with

SMCC or sslinking agent. This boost is repeated five days (IP), four days (IP), three days (IP) and one day (IV) prior to fusion. The mouse spleen cells are then fused to myeloma (e.g., 653) cells at a ratio of 1:1 using PEG 1500 (Boehringer Mannheim), and the cell fusion is plated and screened for OP-1-specific antibodies using OP-1 (307-431) as antigen. The cell fusion and monoclonal screening are according to procedures widely available in the art. The neutralizing monoclonal is identified by its ability to block the biological activity of OP-1 when added to a cellular assay which responds biologically to added OP-1.

8. Identification of OP-1 Producing Cell Line Which Displays OP-1 Surface Receptors

During the process of routinely testing the effects of increasing concentrations of OP-1 and TGF- β on the proliferation of various cell lines, a cell line was identified which, surprising, appears not only to synthesize and secrete OP-1, but also to display cell surface receptors to which the secreted OP-1 binds and acts to inhibit proliferation of the cells. This cell line was identified after the following observations. Addition of increasing concentrations of OP-1 or TGF- β failed to increase or decrease the relatively low basal rate of proliferation of the cells. However, addition of a monoclonal antibody, which neutralizes the activity of Op-1, resulted in a large increase in the proliferation of the cells. In addition, simultaneous addition of the same quantity of OP-1 neutralizing monoclonal to a fixed amount of OP-1 resulted in an increase in proliferation which was intermediate between the low

basal level observed with OP-1 alone and the high level observed with the monoclonal alone. This cell line, which is an epithelial cell line that was derived from a bladder cell carcinoma, may be used in an assay of the invention. The parameter to be tested according to the invention is cellular proliferation. Thus, a compound(s) that increases or decreases the level of OP-1 production may be tested on this cell line as follows..

9. Assay for Identifying Drugs Which Affect OP-1 Synthesis

A simple medium flux screening assay can be configured in a standard 24 or 96 well microtiter dish, in which each well contains a constant number of a cell line having the characteristics described above. Increasing concentrations of an OP-1 neutralizing monoclonal antibody is added from left to right across the dish. A constant amount of different test substances is added from top to bottom on the dish. An increase in the synthesis and secretion of OP-1 (over its constitutive (non-induced) level) will be indicated by an increase in the amount of OP-1 neutralizing antibody required to release the cells from the antimitogenic activity of OP-1. A decrease in the synthesis and secretion of OP-1 (below its constitutive (repressed) level) will be indicated by the observation that decreased concentrations of the OP-1 neutralizing monoclonal antibody will be required to release the cells from the antimitogenic activity of OP-1. One of the major advantages of this assay is that the end point, i.e., the dilution of antibody which has an effect on cell proliferation, is a measure of mitosis, or an increase in

the number of cells per well. Because several convenient and rapid assays exist for quantitating cell numbers, this assay is faster and requires significantly fewer steps to perform.

The assay may be performed as follows. After addition of appropriate concentrations of the OP-1 neutralizing monoclonal antibody and test substances to the wells containing the cells, the dishes are placed in an incubator at 37°C for a period of 1-3 days. After completion of incubation/growth period, the dishes are removed and the cells in the individual wells are washed and stained with a vital stain, such as crystal violet. Washing and staining procedures are well-known in the art. The cells are then lysed and the stain dissolved in a constant amount of a solvent, such as ethanol. Quantitations of the dissolved stain, which is readily performed on an automated plate vendor, allows for direct quantitation of the number of cells in each well.

The above-described assay has the advantages of being rapid and easy to perform because it requires few steps. Another advantage is intrinsic to the assay; drugs which are screened according to this procedure that result in cell death (i.e., cytotoxic substances) are immediately, identifiable without the need of operator observation. In addition, although drugs that stop the growth of the cells (i.e., cytostatic substances) are scored as positive due to failure to see increases in cell numbers, they are automatically scored as suspect due to the failure of the highest concentrations of OP-1 neutralizing monoclonal antibody to release the cells from the antimitogenic activity of OP-1.

10. Candidate Drugs to Screen

The screening methods of the invention is used to test compounds for their effect on the production of morphogenic protein by a given cell type. Examples of compounds which may be screened include but are not limited to chemicals, biological response modifiers (e.g., lymphokines, cytokines, hormones, or vitamins), plant extracts, microbial broths and extracts medium conditioned by eukaryotic cells, body fluids, or tissue extracts.

The invention may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The present embodiments are therefore to be considered in all respects as illustrative and not restrictive, the scope of the invention being indicated by the appended claims rather than by the foregoing description, and all changes which come within the meaning and range of equivalency of the claims are therefore intended to be embraced therein.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: John Smart
Herman Oppermann
Engin Ozkaynak
Thangavel Kuberasampath
David C. Rueger
Roy H.L. Pang
Charles M. Cohen
- (ii) TITLE OF INVENTION: MORPHOGENIC
PROTEIN SCREENING METHOD
- (iii) NUMBER OF SEQUENCES: 33
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: Creative BioMolecules
 - (B) STREET: 35 South Street
 - (C) CITY: Hopkinton
 - (D) STATE: Massachusetts
 - (E) COUNTRY: U.S.A.
 - (F) ZIP: 01748
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Diskette, 5.25,
360kb storage
 - (B) COMPUTER: IBM XT
 - (C) OPERATING SYSTEM: DOS 3.30
 - (D) SOFTWARE: ASC II TEXT
- (vi) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: US 667,274
 - (B) FILING DATE: March 11, 1991
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: US 752,861
 - (B) FILING DATE: AUGUST 30, 1991

(viii) ATTORNEY/AGENT INFORMATION

(A) NAME: PITCHER, EDMUND R.

(B) REG. NO.: 27,829

(C) DOCKET NO.: CRP-058^c₄PC

(ix) TELEPHONE:

(A) 617/248-7000

(B) TELEFAX: 617/248-7100

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 97 amino acids

(B) TYPE: amino acids

(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

(A) NAME: Generic Sequence 1

(D) OTHER INFORMATION: Each Xaa indicates one of the 20 naturally-occurring L-isomer, α -amino acids or a derivative thereof.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```

Xaa Xaa Xaa Xaa Xaa Xaa
 1                      5
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
 10                      15
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Xaa Xaa
 20                      25
Cys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
 30                      35
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
 40                      45                      50
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
                      55                      60
Cys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
                      65                      70
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
                      75                      80
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
                      85                      90
Xaa Cys Xaa
95

```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 97 amino acids

(B) TYPE: amino acids

(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

(A) NAME: Generic Sequence 2

(D) OTHER INFORMATION: Each Xaa indicates one of the 20 naturally-occurring L-isomer, α -amino acids or a derivative thereof.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```

Xaa Xaa Xaa Xaa Xaa Xaa
      1                      5
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
      10                      15
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Xaa Xaa
      20                      25
Cys Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Xaa Xaa
      30                      35
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
      40                      45                      50
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
      55                      60
Cys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
      65                      70
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
      75                      80
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
      85                      90
Xaa Cys Xaa
      95

```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 97 amino acids

(B) TYPE: amino acids

(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

(A) NAME: Generic Sequence 3

(D) OTHER INFORMATION: wherein each
Xaa is independently selected from
a group of one or more specified
amino acids as defined in the
specification.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```

      Leu Tyr Val Xaa Phe
      1             5
Xaa Xaa Xaa Gly Trp Xaa Xaa Trp Xaa
      10
Xaa Ala Pro Gly Xaa Xaa Xaa Ala
      15             20
Xaa Tyr Cys Xaa Gly Xaa Cys Xaa
      25             30
Xaa Pro Xaa Xaa Xaa Xaa Xaa
      35
Xaa Xaa Xaa Asn His Ala Xaa Xaa
      40             45
Xaa Xaa Leu Xaa Xaa Xaa Xaa Xaa
      50
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
      55             60
Cys Xaa Pro Xaa Xaa Xaa Xaa Xaa
      65

```

Xaa Xaa Xaa L u Xaa Xaa Xaa
 70 75
 Xaa Xaa Xaa Xaa Val Xaa Leu Xaa
 80
 Xaa Xaa Xaa Xaa Met Xaa Val Xaa
 85 90
 Xaa Cys Gly Cys Xaa
 95

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 102 amino acids
 - (B) TYPE: amino acids
 - (C) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (ix) FEATURE:
 - (A) NAME: Generic Sequence 4
 - (D) OTHER INFORMATION: wherein each Xaa is independently selected from a group of one or more specified amino acids as defined in the specification.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Cys Xaa Xaa Xaa Xaa Leu Tyr Val Xaa Phe
 1 5 10
 Xaa Xaa Xaa Gly Trp Xaa Xaa Trp Xaa
 15
 Xaa Ala Pro Xaa Gly Xaa Xaa Ala
 20 25
 Xaa Tyr Cys Xaa Gly Xaa Cys Xaa
 30 35
 Xaa Pro Xaa Xaa Xaa Xaa Xaa
 40

```

Asn Xaa Xaa Asn His Ala Xaa Xaa
      45                      50
Xaa Xaa Leu Xaa Xaa Xaa Xaa Xaa
      55
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
      60                      65
Cys Xaa Pro Xaa Xaa Xaa Xaa Xaa
      70
Xaa Xaa Xaa Leu Xaa Xaa Xaa
      75                      80
Xaa Xaa Xaa Xaa Val Xaa Leu Xaa
      85
Xaa Xaa Xaa Xaa Met Xaa Val Xaa
      90                      95
Xaa Cys Gly Cys Xaa
      100

```

- (2) INFORMATION FOR SEQ ID NO:5:
- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 139 amino acids
 - (B) TYPE: amino acids
 - (C) TOPOLOGY: linear
 - (ii) MOLECULE TYPE: protein
 - (ix) FEATURE:
 - (A) NAME: hOP-1 (mature form)
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```

Ser Thr Gly Ser Lys Gln Arg Ser Gln
  1                      5
Asn Arg Ser Lys Thr Pro Lys Asn Gln
 10                      15
Glu Ala Leu Arg Met Ala Asn Val Ala
    20                      25
Glu Asn Ser Ser Ser Asp Gln Arg Gln
    30                      35

```

| | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ala | Cys | Lys | Lys | His | Glu | Leu | Tyr | Val |
| | | | 40 | | | | | 45 |
| Ser | Phe | Arg | Asp | Leu | Gly | Trp | Gln | Asp |
| | | | 50 | | | | | |
| Trp | Ile | Ile | Ala | Pro | Glu | Gly | Tyr | Ala |
| 55 | | | | | 60 | | | |
| Ala | Tyr | Tyr | Cys | Glu | Gly | Glu | Cys | Ala |
| | 65 | | | | | 70 | | |
| Phe | Pro | Leu | Asn | Ser | Tyr | Met | Asn | Ala |
| | | 75 | | | | | 80 | |
| Thr | Asn | His | Ala | Ile | Val | Gln | Thr | Leu |
| | | | 85 | | | | | 90 |
| Val | His | Phe | Ile | Asn | Pro | Glu | Thr | Val |
| | | | 95 | | | | | |
| Pro | Lys | Pro | Cys | Cys | Ala | Pro | Thr | Gln |
| 100 | | | | | 105 | | | |
| Leu | Asn | Ala | Ile | Ser | Val | Leu | Tyr | Phe |
| | 110 | | | | | 115 | | |
| Asp | Asp | Ser | Ser | Asn | Val | Ile | Leu | Lys |
| | | 120 | | | | | 125 | |
| Lys | Tyr | Arg | Asn | Met | Val | Val | Arg | Ala |
| | | | 130 | | | | | 135 |
| Cys | Gly | Cys | His | | | | | |

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 139 amino acids

(B) TYPE: amino acids

(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

(A) NAME: mOP-1 (mature form)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

| | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ser | Thr | Gly | Gly | Lys | Gln | Arg | Ser | Gln |
| 1 | | | | 5 | | | | |
| Asn | Arg | Ser | Lys | Thr | Pro | Lys | Asn | Gln |
| 10 | | | | | 15 | | | |
| Glu | Ala | Leu | Arg | Met | Ala | Ser | Val | Ala |
| | 20 | | | | | 25 | | |
| Glu | Asn | Ser | Ser | Ser | Asp | Gln | Arg | Gln |
| | | 30 | | | | | 35 | |
| Ala | Cys | Lys | Lys | His | Glu | Leu | Tyr | Val |
| | | | 40 | | | | | 45 |
| Ser | Phe | Arg | Asp | Leu | Gly | Trp | Gln | Asp |
| | | | | 50 | | | | |
| Trp | Ile | Ile | Ala | Pro | Glu | Gly | Tyr | Ala |
| 55 | | | | | 60 | | | |
| Ala | Tyr | Tyr | Cys | Glu | Gly | Glu | Cys | Ala |
| | 65 | | | | | 70 | | |
| Phe | Pro | Leu | Asn | Ser | Tyr | Met | Asn | Ala |
| | | 75 | | | | | 80 | |
| Thr | Asn | His | Ala | Ile | Val | Gln | Thr | Leu |
| | | | 85 | | | | | 90 |
| Val | His | Phe | Ile | Asn | Pro | Asp | Thr | Val |
| | | | | 95 | | | | |
| Pro | Lys | Pro | Cys | Cys | Ala | Pro | Thr | Gln |
| 100 | | | | | 105 | | | |
| Leu | Asn | Ala | Ile | Ser | Val | Leu | Tyr | Phe |
| | 110 | | | | | | 115 | |

| | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Asp | Asp | Ser | Ser | Asn | Val | Ile | Leu | Lys |
| | | 120 | | | | | 125 | |
| Lys | Tyr | Arg | Asn | Met | Val | Val | Arg | Ala |
| | | | 130 | | | | | 135 |
| Cys | Gly | Cys | His | | | | | |

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 139 amino acids

(B) TYPE: amino acids

(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

(A) NAME: hOP-2 (mature form)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

| | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ala | Val | Arg | Pro | Leu | Arg | Arg | Arg | Gln |
| 1 | | | | 5 | | | | |
| Pro | Lys | Lys | Ser | Asn | Glu | Leu | Pro | Gln |
| 10 | | | | | 15 | | | |
| Ala | Asn | Arg | Leu | Pro | Gly | Ile | Phe | Asp |
| 20 | | | | | | 25 | | |
| Asp | Val | His | Gly | Ser | His | Gly | Arg | Gln |
| 30 | | | | | | | 35 | |
| Val | Cys | Arg | Arg | His | Glu | Leu | Tyr | Val |
| | | | 40 | | | | | 45 |
| Ser | Phe | Gln | Asp | Leu | Gly | Trp | Leu | Asp |
| | | | | 50 | | | | |
| Trp | Val | Ile | Ala | Pro | Gln | Gly | Tyr | Ser |
| 55 | | | | | 60 | | | |
| Ala | Tyr | Tyr | Cys | Glu | Gly | Glu | Cys | Ser |
| 65 | | | | | | 70 | | |
| Phe | Pro | Leu | Asp | Ser | Cys | Met | Asn | Ala |
| | | | 75 | | | | 80 | |
| Thr | Asn | His | Ala | Ile | Leu | Gln | Ser | Leu |
| | | | 85 | | | | | 90 |

| | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|
| Val | His | Leu | Met | Lys | Pro | Asn | Ala | Val | |
| | | | | 95 | | | | | |
| Pro | Lys | Ala | Cys | Cys | Ala | Pro | Thr | Lys | |
| 100 | | | | | 105 | | | | |
| Leu | Ser | Ala | Thr | Ser | Val | Leu | Tyr | Tyr | |
| | 110 | | | | | 115 | | | |
| Asp | Ser | Ser | Asn | Asn | Val | Ile | Leu | Arg | |
| | | 120 | | | | | 125 | | |
| Lys | His | Arg | Asn | Met | Val | Val | Lys | Ala | |
| | | | 130 | | | | | 135 | |
| Cys | Gly | Cys | His | | | | | | |

- (2) INFORMATION FOR SEQ ID NO:8:
- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 139 amino acids
 - (B) TYPE: amino acids
 - (C) TOPOLOGY: linear
 - (ii) MOLECULE TYPE: protein
 - (ix) FEATURE:
 - (A) NAME: mOP-2 (mature form)
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

| | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|
| Ala | Ala | Arg | Pro | Leu | Lys | Arg | Arg | Gln | |
| 1 | | | | 5 | | | | | |
| Pro | Lys | Lys | Thr | Asn | Glu | Leu | Pro | His | |
| 10 | | | | | 15 | | | | |
| Pro | Asn | Lys | Leu | Pro | Gly | Ile | Phe | Asp | |
| | 20 | | | | | 25 | | | |
| Asp | Gly | His | Gly | Ser | Arg | Gly | Arg | Glu | |
| | | 30 | | | | | 35 | | |
| Val | Cys | Arg | Arg | His | Glu | Leu | Tyr | Val | |
| | | | 40 | | | | | 45 | |
| Ser | Phe | Arg | Asp | Leu | Gly | Trp | Leu | Asp | |
| | | | | 50 | | | | | |
| Trp | Val | Ile | Ala | Pro | Gln | Gly | Tyr | Ser | |
| 55 | | | | | 60 | | | | |

| | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ala | Tyr | Tyr | Cys | Glu | Gly | Glu | Cys | Ala |
| | 65 | | | | | 70 | | |
| Phe | Pro | Leu | Asp | Ser | Cys | Met | Asn | Ala |
| | | 75 | | | | | 80 | |
| Thr | Asn | His | Ala | Ile | Leu | Gln | Ser | Leu |
| | | | 85 | | | | | 90 |
| Val | His | Leu | Met | Lys | Pro | Asp | Val | Val |
| | | | | 95 | | | | |
| Pro | Lys | Ala | Cys | Cys | Ala | Pro | Thr | Lys |
| 100 | | | | | 105 | | | |
| Leu | Ser | Ala | Thr | Ser | Val | Leu | Tyr | Tyr |
| | 110 | | | | | 115 | | |
| Asp | Ser | Ser | Asn | Asn | Val | Ile | Leu | Arg |
| | | 120 | | | | | 125 | |
| Lys | His | Arg | Asn | Met | Val | Val | Lys | Ala |
| | | | 130 | | | | | 135 |
| Cys | Gly | Cys | His | | | | | |

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 96 amino acids

(B) TYPE: amino acids

(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

(A) NAME: CBMP-2A(fx)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Cys Lys Arg His Pro Leu Tyr Val Asp Phe Ser
 1 5 10
 Asp Val Gly Trp Asn Asp Trp Ile Val Ala Pro
 15 20
 Pro Gly Tyr His Ala Phe Tyr Cys His Gly Glu
 25 30
 Cys Pro Phe Pro Leu Ala Asp His Leu Asn Ser
 35 40
 Thr Asn His Ala Ile Val Gln Thr Leu Val Asn
 45 50 55
 Ser Val Asn Ser Lys Ile Pro Lys Ala Cys Cys
 60 65
 Val Pro Thr Glu Leu Ser Ala Ile Ser Met Leu
 70 75
 Tyr Leu Asp Glu Asn Glu Lys Val Val Leu Lys
 80 85
 Asn Tyr Gln Asp Met Val Val Glu Gly Cys Gly
 90 95
 Cys Arg
 100

(2) INFORMATION FOR SEQ ID NO:10:

(i) **SEQUENCE CHARACTERISTICS:**

(A) LENGTH: 101 amino acids

(B) TYPE: amino acids

(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

(A) NAME: CBMP-2B(fx)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

| | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|
| | | | | | | | Cys | Arg | Arg | His | Ser | |
| | | | | | | | 1 | | | | 5 | |
| Leu | Tyr | Val | Asp | Phe | Ser | Asp | Val | Gly | Trp | Asn | | |
| | | | | 10 | | | | | | 15 | | |
| Asp | Trp | Ile | Val | Ala | Pro | Pro | Gly | Tyr | Gln | Ala | | |
| | | | 20 | | | | | | 25 | | | |
| Phe | Tyr | Cys | His | Gly | Asp | Cys | Pro | Phe | Pro | Leu | | |
| | | | 30 | | | | | 35 | | | | |
| Ala | Asp | His | Leu | Asn | Ser | Thr | Asn | His | Ala | Ile | | |
| | 40 | | | | | 45 | | | | | | |
| Val | Gln | Thr | Leu | Val | Asn | Ser | Val | Asn | Ser | Ser | | |
| 50 | | | | | 55 | | | | | 60 | | |
| Ile | Pro | Lys | Ala | Cys | Cys | Val | Pro | Thr | Glu | Leu | | |
| | | | | 65 | | | | | | 70 | | |
| Ser | Ala | Ile | Ser | Met | Leu | Tyr | Leu | Asp | Glu | Tyr | | |
| | | | 75 | | | | | 80 | | | | |
| Asp | Lys | Val | Val | Leu | Lys | Asn | Tyr | Gln | Glu | Met | | |
| | | 85 | | | | | 90 | | | | | |
| Val | Val | Glu | Gly | Cys | Gly | Cys | Arg | | | | | |
| 95 | | | | | | 100 | | | | | | |

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 102 amino acids

(B) TYPE: amino acids

(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

(A) NAME: DPP(fx)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```

Cys Arg Arg His Ser Leu Tyr Val Asp Phe Ser
 1             5             10
Asp Val Gly Trp Asp Asp Trp Ile Val Ala Pro
          15             20
Leu Gly Tyr Asp Ala Tyr Tyr Cys His Gly Lys
          25             30
Cys Pro Phe Pro Leu Ala Asp His Phe Asn Ser
          35             40
Thr Asn His Ala Val Val Gln Thr Leu Val Asn
 45             50             55
Asn Asn Asn Pro Gly Lys Val Pro Lys Ala Cys
          60             65
Cys Val Pro Thr Gln Leu Asp Ser Val Ala Met
          70             75
Leu Tyr Leu Asn Asp Gln Ser Thr Val Val Leu
          80             85
Lys Asn Tyr Gln Glu Met Thr Val Val Gly Cys
          90             95
Gly Cys Arg
100

```

- (2) INFORMATION FOR SEQ ID NO:12:
- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 102 amino acids
 - (B) TYPE: amino acids
 - (C) TOPOLOGY: linear
 - (ii) MOLECULE TYPE: protein
 - (ix) FEATURE:
 - (A) NAME: Vgl(fx)
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

```

Cys Lys Lys Arg His Leu Tyr Val Glu Phe Lys
 1             5             10
Asp Val Gly Trp Gln Asn Trp Val Ile Ala Pro
      15             20
Gln Gly Tyr Met Ala Asn Tyr Cys Tyr Gly Glu
      25             30
Cys Pro Tyr Pro Leu Thr Glu Ile Leu Asn Gly
      35             40
Ser Asn His Ala Ile Leu Gln Thr Leu Val His
      45             50             55
Ser Ile Glu Pro Glu Asp Ile Pro Leu Pro Cys
      60             65
Cys Val Pro Thr Lys Met Ser Pro Ile Ser Met
      70             75
Leu Phe Tyr Asp Asn Asn Asp Asn Val Val Leu
      80             85
Arg His Tyr Glu Asn Met Ala Val Asp Glu Cys
      90             95
Gly Cys Arg
100

```


- (2) INFORMATION FOR SEQ ID NO:13:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 102 amino acids
- (B) TYPE: amino acids
- (C) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (ix) FEATURE:
- (A) NAME: Vgr-1(fx)
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:
- Cys Lys Lys His Glu Leu Tyr Val Ser Phe Gln
 1 5 10
 Asp Val Gly Trp Gln Asp Trp Ile Ile Ala Pro
 15 20
 Xaa Gly Tyr Ala Ala Asn Tyr Cys Asp Gly Glu
 25 30
 Cys Ser Phe Pro Leu Asn Ala His Met Asn Ala
 35 40
 Thr Asn His Ala Ile Val Gln Thr Leu Val His
 45 50 55
 Val Met Asn Pro Glu Tyr Val Pro Lys Pro Cys
 60 65
 Cys Ala Pro Thr Lys Val Asn Ala Ile Ser Val
 70 75
 Leu Tyr Phe Asp Asp Asn Ser Asn Val Ile Leu
 80 85
 Lys Lys Tyr Arg Asn Met Val Val Arg Ala Cys
 90 95
 Gly Cys His
 100

(2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 106 amino acids
- (B) TYPE: protein
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: human
- (F) TISSUE TYPE: BRAIN

(ix) FEATURE:

- (D) OTHER INFORMATION:
/product= "GDF-1 (fx)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

```

Cys Arg Ala Arg Arg Leu Tyr Val Ser Phe Arg Glu Val Gly
 1             5             10
Trp His Arg Trp Val Ile Ala Pro Arg Gly Phe Leu Ala Asn Tyr
15             20             25
Cys Gln Gly Gln Cys Ala Leu Pro Val Ala Leu Ser Gly Ser Gly
30             35             40
Gly Pro Pro Ala Leu Asn His Ala Val Leu Arg Ala Leu Met His
45             50             55
Ala Ala Ala Pro Gly Ala Ala Asp Leu Pro Cys Cys Val Pro Ala
60             65             70
Arg Leu Ser Pro Ile Ser Val Leu Phe Phe Asp Asn Ser Asp Asn
75             80             85
Val Val Leu Arg Gln Tyr Glu Asp Met Val Val Asp Glu Cys Gly
90             95             100
Cys Arg
105

```

(2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 5 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Cys Xaa Xaa Xaa Xaa
 1 5

(2) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1822 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: HOMO SAPIENS
 (F) TISSUE TYPE: HIPPOCAMPUS

(ix) FEATURE:

- (A) NAME/KEY: CDS
 (B) LOCATION: 49..1341
 (D) OTHER INFORMATION: /standard_name= "hOP1"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

```

GGTGC GGGGCC CGGAGCCCGG AGCCCGGGTA GCGCGTAGAG CCGGCGCG ATG CAC GTG      57
                                         Met His Val
                                         1

CGC TCA CTG CGA GCT GCG GCG CCG CAC AGC TTC GTG GCG CTC TGG GCA      105
Arg Ser Leu Arg Ala Ala Ala Pro His Ser Phe Val Ala Leu Trp Ala
      5              10              15

CCC CTG TTC CTG CTG CGC TCC GCC CTG GCC GAC TTC AGC CTG GAC AAC      153
Pro Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser Leu Asp Asn
      20              25              30              35

GAG GTG CAC TCG AGC TTC ATC CAC CGG CGC CTC CGC AGC CAG GAG CGG      201
Glu Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser Gln Glu Arg
              40              45              50

CGG GAG ATG CAG CGC GAG ATC CTC TCC ATT TTG GGC TTG CCC CAC CGC      249
Arg Glu Met Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu Pro His Arg
              55              60              65

```

| | |
|---|-----|
| CCG CGC CCG CAC CTC CAG GGC AAG CAC AAC TCG GCA CCC ATG TTC ATG Pro Arg Pro His Leu Gln Gly Lys His Asn Ser Ala Pro Met Phe Met 70 75 80 | 297 |
| CTG GAC CTG TAC AAC GCC ATG GCG GTG GAG GAG GGC GGC GGG CCC GGC Leu Asp Leu Tyr Asn Ala Met Ala Val Glu Glu Gly Gly Gly Pro Gly 85 90 95 | 345 |
| GGC CAG GGC TTC TCC TAC CCC TAC AAG GCC GTC TTC AGT ACC CAG GGC Gly Gln Gly Phe Ser Tyr Pro Tyr Lys Ala Val Phe Ser Thr Gln Gly 100 105 110 115 | 393 |
| CCC CCT CTG GCC AGC CTG CAA GAT AGC CAT TTC CTC ACC GAC GCC GAC Pro Pro Leu Ala Ser Leu Gln Asp Ser His Phe Leu Thr Asp Ala Asp 120 125 130 | 441 |
| ATG GTC ATG AGC TTC GTC AAC CTC GTG GAA CAT GAC AAG GAA TTC TTC Met Val Met Ser Phe Val Asn Leu Val Glu His Asp Lys Glu Phe Phe 135 140 145 | 489 |
| CAC CCA CGC TAC CAC CAT CGA GAG TTC CGG TTT GAT CTT TCC AAG ATC His Pro Arg Tyr His His Arg Glu Phe Arg Phe Asp Leu Ser Lys Ile 150 155 160 | 537 |
| CCA GAA GGC GAA GCT GTC ACG GCA GCC GAA TTC CGG ATC TAC AAG GAC Pro Glu Gly Glu Ala Val Thr Ala Ala Glu Phe Arg Ile Tyr Lys Asp 165 170 175 | 585 |
| TAC ATC CGG GAA CGC TTC GAC AAT GAG ACG TTC CGG ATC AGC GTT TAT Tyr Ile Arg Glu Arg Phe Asp Asn Glu Thr Phe Arg Ile Ser Val Tyr 180 185 190 195 | 633 |
| CAG GTG CTC CAG GAG CAC TTG GGC AGG GAA TCG GAT CTC TTC CTG CTC Gln Val Leu Gln Glu His Leu Gly Arg Glu Ser Asp Leu Phe Leu Leu 200 205 210 | 681 |
| GAC AGC CGT ACC CTC TGG GCC TCG GAG GAG GGC TGG CTG GTG TTT GAC Asp Ser Arg Thr Leu Trp Ala Ser Glu Glu Gly Trp Leu Val Phe Asp 215 220 225 | 729 |
| ATC ACA GCC ACC AGC AAC CAC TGG GTG GTC AAT CCG CGG CAC AAC CTG Ile Thr Ala Thr Ser Asn His Trp Val Val Asn Pro Arg His Asn Leu 230 235 240 | 777 |
| GGC CTG CAG CTC TCG GTG GAG ACG CTG GAT GGG CAG AGC ATC AAC CCC Gly Leu Gln Leu Ser Val Glu Thr Leu Asp Gly Gln Ser Ile Asn Pro 245 250 255 | 825 |
| AAG TTG GCG GGC CTG ATT GGG CGG CAC GGG CCC CAG AAC AAG CAG CCC Lys Leu Ala Gly Leu Ile Gly Arg His Gly Pro Gln Asn Lys Gln Pro 260 265 270 275 | 873 |

| | |
|---|------|
| TTC ATG GTG GCT TTC TTC AAG GCC ACG GAG GTC CAC TTC CGC AGC ATC Phe Met Val Ala Phe Phe Lys Ala Thr Glu Val His Phe Arg Ser Ile 280 285 290 | 921 |
| CGG TCC ACG GGG AGC AAA CAG CGC AGC CAG AAC CGC TCC AAG ACG CCC Arg Ser Thr Gly Ser Lys Gln Arg Ser Gln Asn Arg Ser Lys Thr Pro 295 300 305 | 969 |
| AAG AAC CAG GAA GCC CTG CGG ATG GCC AAC GTG GCA GAG AAC AGC AGC Lys Asn Gln Glu Ala Leu Arg Met Ala Asn Val Ala Glu Asn Ser Ser 310 315 320 | 1017 |
| AGC GAC CAG AGG CAG GCC TGT AAG AAG CAC GAG CTG TAT GTC AGC TTC Ser Asp Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr Val Ser Phe 325 330 335 | 1065 |
| CGA GAC CTG GGC TGG CAG GAC TGG ATC ATC GCG CCT GAA GGC TAC GCC Arg Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala 340 345 350 355 | 1113 |
| GCC TAC TAC TGT GAG GGG GAG TGT GCC TTC CCT CTG AAC TCC TAC ATG Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn Ser Tyr Met 360 365 370 | 1161 |
| AAC GCC ACC AAC CAC GCC ATC GTG CAG ACG CTG GTC CAC TTC ATC AAC Asn Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His Phe Ile Asn 375 380 385 | 1209 |
| CCG GAA ACG GTG CCC AAG CCC TGC TGT GCG CCC ACG CAG CTC AAT GCC Pro Glu Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln Leu Asn Ala 390 395 400 | 1257 |
| ATC TCC GTC CTC TAC TTC GAT GAC AGC TCC AAC GTC ATC CTG AAG AAA Ile Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile Leu Lys Lys 405 410 415 | 1305 |
| TAC AGA AAC ATG GTG GTC CGG GCC TGT GGC TGC CAC TAGCTCCTCC Tyr Arg Asn Met Val Val Arg Ala Cys Gly Cys His 420 425 430 | 1351 |
| GAGAATTCAG ACCCTTTGGG GCCAAGTTTT TCTGGATCCT CCATTGCTCG CCTTGGCCAG | 1411 |
| GAACCAGCAG ACCAACTGCC TTTTGTGAGA CCTTCCCCTC CCTATCCCCA ACTTTAAAGG | 1471 |
| TGTGAGAGTA TTAGGAAACA TGAGCAGCAT ATGGCTTTTG ATCAGTTTTT CAGTGGCAGC | 1531 |
| ATCCAATGAA CAAGATCCTA CAAGCTGTGC AGGCAAAACC TAGCAGGAAA AAAAAACAAC | 1591 |
| GCATAAAGAA AAATGGCCGG GCCAGGTCAT TGGCTGGGAA GTCTCAGCCA TGCACGGACT | 1651 |
| CGTTTCCAGA GGTAAATTATG AGCGCCTACC AGCCAGGCCA CCCAGCCGTG GGAGGAAGGG | 1711 |
| GGCGTGGCAA GGGGTGGGCA CATTGGTGTC TGTGCGAAAG GAAAATTGAC CCGGAAGTTC | 1771 |
| CTGTAATAAAA TGTCAACAATA AAACGAATGA ATGAAAAAAAA AAAAAAAAAA A | 1822 |

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 431 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

(D) OTHER INFORMATION: /Product="OP1-PP"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

```

Met His Val Arg Ser Leu Arg Ala Ala Ala Pro His Ser Phe Val Ala
 1           5           10           15
Leu Trp Ala Pro Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser
          20           25           30
Leu Asp Asn Glu Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser
          35           40           45
Gln Glu Arg Arg Glu Met Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu
          50           55           60
Pro His Arg Pro Arg Pro His Leu Gln Gly Lys His Asn Ser Ala Pro
          65           70           75           80
Met Phe Met Leu Asp Leu Tyr Asn Ala Met Ala Val Glu Glu Gly Gly
          85           90           95
Gly Pro Gly Gly Gln Gly Phe Ser Tyr Pro Tyr Lys Ala Val Phe Ser
          100          105          110
Thr Gln Gly Pro Pro Leu Ala Ser Leu Gln Asp Ser His Phe Leu Thr
          115          120          125
Asp Ala Asp Met Val Met Ser Phe Val Asn Leu Val Glu His Asp Lys
          130          135          140
Glu Phe Phe His Pro Arg Tyr His His Arg Glu Phe Arg Phe Asp Leu
          145          150          155          160
Ser Lys Ile Pro Glu Gly Glu Ala Val Thr Ala Ala Glu Phe Arg Ile
          165          170          175
Tyr Lys Asp Tyr Ile Arg Glu Arg Phe Asp Asn Glu Thr Phe Arg Ile
          180          185          190
Ser Val Tyr Gln Val Leu Gln Glu His Leu Gly Arg Glu Ser Asp Leu
          195          200          205

```

Phe Leu Leu Asp Ser Arg Thr Leu Trp Ala Ser Glu Glu Gly Trp Leu
 210 215 220
 Val Phe Asp Ile Thr Ala Thr Ser Asn His Trp Val Val Asn Pro Arg
 225 230 235 240
 His Asn Leu Gly Leu Gln Leu Ser Val Glu Thr Leu Asp Gly Gln Ser
 245 250 255
 Ile Asn Pro Lys Leu Ala Gly Leu Ile Gly Arg His Gly Pro Gln Asn
 260 265 270
 Lys Gln Pro Phe Met Val Ala Phe Phe Lys Ala Thr Glu Val His Phe
 275 280 285
 Arg Ser Ile Arg Ser Thr Gly Ser Lys Gln Arg Ser Gln Asn Arg Ser
 290 295 300
 Lys Thr Pro Lys Asn Gln Glu Ala Leu Arg Met Ala Asn Val Ala Glu
 305 310 315 320
 Asn Ser Ser Ser Asp Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr
 325 330 335
 Val Ser Phe Arg Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu
 340 345 350
 Gly Tyr Ala Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn
 355 360 365
 Ser Tyr Met Asn Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His
 370 375 380
 Phe Ile Asn Pro Glu Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln
 385 390 395 400
 Leu Asn Ala Ile Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile
 405 410 415
 Leu Lys Lys Tyr Arg Asn Met Val Val Arg Ala Cys Gly Cys His
 420 425 430

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1873 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: MURIDAE
- (F) TISSUE TYPE: EMBRYO

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 104..1393
- (D) OTHER INFORMATION: /note= "MOP1 (CDNA)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

| | |
|---|-----|
| CTGCAGCAAG TGACCTCGGG TCGTGGACCG CTGCCCTGCC CCCTCCGCTG CCACCTGGGG | 60 |
| CGGCGCGGGC CCGGTGCCCC GGATCGCGCG TAGAGCCGGC GCG ATG CAC GTG CGC | 115 |
| Met His Val Arg | |
| 1 | |
| TCG CTG CGC GCT GCG GCG CCA CAC AGC TTC GTG GCG CTC TGG GCG CCT | 163 |
| Ser Leu Arg Ala Ala Ala Pro His Ser Phe Val Ala Leu Trp Ala Pro | |
| 5 10 15 20 | |
| CTG TTC TTG CTG CGC TCC GCC CTG GCC GAT TTC AGC CTG GAC AAC GAG | 211 |
| Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser Leu Asp Asn Glu | |
| 25 30 35 | |
| GTG CAC TCC AGC TTC ATC CAC CGG CGC CTC CGC AGC CAG GAG CGG CGG | 259 |
| Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser Gln Glu Arg Arg | |
| 40 45 50 | |
| GAG ATG CAG CGG GAG ATC CTG TCC ATC TTA GGG TTG CCC CAT CGC CCG | 307 |
| Glu Met Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu Pro His Arg Pro | |
| 55 60 65 | |
| CGC CCG CAC CTC CAG GGA AAG CAT AAT TCG GCG CCC ATG TTC ATG TTG | 355 |
| Arg Pro His Leu Gln Gly Lys His Asn Ser Ala Pro Met Phe Met Leu | |
| 70 75 80 | |
| GAC CTG TAC AAC GCC ATG GCG GTG GAG GAG AGC GGG CCG GAC GGA CAG | 403 |
| Asp Leu Tyr Asn Ala Met Ala Val Glu Glu Ser Gly Pro Asp Gly Gln | |
| 85 90 95 100 | |

| | |
|---|------|
| GGC TTC TCC TAC CCC TAC AAG GCC GTC TTC AGT ACC CAG GGC CCC CCT Gly Phe Ser Tyr Pro Tyr Lys Ala Val Phe Ser Thr Gln Gly Pro Pro 105 110 115 | 451 |
| TTA GCC AGC CTG CAG GAC AGC CAT TTC CTC ACT GAC GCC GAC ATG GTC Leu Ala Ser Leu Gln Asp Ser His Phe Leu Thr Asp Ala Asp Met Val 120 125 130 | 499 |
| ATG AGC TTC GTC AAC CTA GTG GAA CAT GAC AAA GAA TTC TTC CAC CCT Met Ser Phe Val Asn Leu Val Glu His Asp Lys Glu Phe Phe His Pro 135 140 145 | 547 |
| CGA TAC CAC CAT CGG GAG TTC CGG TTT GAT CTT TCC AAG ATC CCC GAG Arg Tyr His His Arg Glu Phe Arg Phe Asp Leu Ser Lys Ile Pro Glu 150 155 160 | 595 |
| GGC GAA CGG GTG ACC GCA GCC GAA TTC AGG ATC TAT AAG GAC TAC ATC Gly Glu Arg Val Thr Ala Glu Phe Arg Ile Tyr Lys Asp Tyr Ile 165 170 175 180 | 643 |
| CGG GAG CGA TTT GAC AAC GAG ACC TTC CAG ATC ACA GTC TAT CAG GTG Arg Glu Arg Phe Asp Asn Glu Thr Phe Gln Ile Thr Val Tyr Gln Val 185 190 195 | 691 |
| CTC CAG GAG CAC TCA GGC AGG GAG TCG GAC CTC TTC TTG CTG GAC AGC Leu Gln Glu His Ser Gly Arg Glu Ser Asp Leu Phe Leu Leu Asp Ser 200 205 210 | 739 |
| CGC ACC ATC TGG GCT TCT GAG GAG GGC TGG TTG GTG TTT GAT ATC ACA Arg Thr Ile Trp Ala Ser Glu Glu Gly Trp Leu Val Phe Asp Ile Thr 215 220 225 | 787 |
| GCC ACC AGC AAC CAC TGG GTG GTC AAC CCT CGG CAC AAC CTG GGC TTA Ala Thr Ser Asn His Trp Val Val Asn Pro Arg His Asn Leu Gly Leu 230 235 240 | 835 |
| CAG CTC TCT GTG GAG ACC CTG GAT GGG CAG AGC ATC AAC CCC AAG TTG Gln Leu Ser Val Glu Thr Leu Asp Gly Gln Ser Ile Asn Pro Lys Leu 245 250 255 260 | 883 |
| GCA GGC CTG ATT GGA CGG CAT GGA CCC CAG AAC AAG CAA CCC TTC ATG Ala Gly Leu Ile Gly Arg His Gly Pro Gln Asn Lys Gln Pro Phe Met 265 270 275 | 931 |
| GTG GCC TTC TTC AAG GCC ACG GAA GTC CAT CTC CGT AGT ATC CGG TCC Val Ala Phe Phe Lys Ala Thr Glu Val His Leu Arg Ser Ile Arg Ser 280 285 290 | 979 |
| ACG GGG GGC AAG CAG CGC AGC CAG AAT CGC TCC AAG ACG CCA AAG AAC Thr Gly Gly Lys Gln Arg Ser Gln Asn Arg Ser Lys Thr Pro Lys Asn 295 300 305 | 1027 |
| CAA GAG GCC CTG AGG ATG GCC AGT GTG GCA GAA AAC AGC AGC AGT GAC Gln Glu Ala Leu Arg Met Ala Ser Val Ala Glu Asn Ser Ser Ser Asp 310 315 320 | 1075 |

| | |
|---|------|
| CAG AGG CAG GCC TGC AAG AAA CAT GAG CTG TAC GTC AGC TTC CGA GAC Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr Val Ser Phe Arg Asp 325 330 335 340 | 1123 |
| CTT GGC TGG CAG GAC TGG ATC ATT GCA CCT GAA GGC TAT GCT GCC TAC Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala Ala Tyr 345 350 355 | 1171 |
| TAC TGT GAG GGA GAG TGC GCC TTC CCT CTG AAC TCC TAC ATG AAC GCC Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn Ser Tyr Met Asn Ala 360 365 370 | 1219 |
| ACC AAC CAC GCC ATC GTC CAG ACA CTG GTT CAC TTC ATC AAC CCA GAC Thr Asn His Ala Ile Val Gln Thr Leu Val His Phe Ile Asn Pro Asp 375 380 385 | 1267 |
| ACA GTA CCC AAG CCC TGC TGT GCG CCC ACC CAG CTC AAC GCC ATC TCT Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln Leu Asn Ala Ile Ser 390 395 400 | 1315 |
| GTC CTC TAC TTC GAC GAC AGC TCT AAT GTC ATC CTG AAG AAG TAC AGA Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile Leu Lys Lys Tyr Arg 405 410 415 420 | 1363 |
| AAC ATG GTG GTC CGG GCC TGT GGC TGC CAC TAGCTCTTCC TGAGACCCTG Asn Met Val Val Arg Ala Cys Gly Cys His 425 430 | 1413 |
| ACCTTTGCGG GGCCACACCT TTCCAAATCT TCGATGTCTC ACCATCTAAG TCTCTCACTG | 1473 |
| CCCACCTTGG CGAGGAGAAC AGACCAACCT CTCCTGAGCC TTCCCTCACC TCCCAACCGG | 1533 |
| AAGCATGTAA GGGTTCCAGA AACCTGAGCG TGCAGCAGCT GATGAGCGCC CTTTCCTTCT | 1593 |
| GGCACGTGAC GGACAAGATC CTACCAGCTA CCACAGCAAA CGCCTAAGAG CAGGAAAAAT | 1653 |
| GTCTGCCAGG AAAGTGTTCCA GTGTCCACAT GGCCCTGGC GCTCTGAGTC TTTGAGGAGT | 1713 |
| AATCGCAAGC CTCGTTGAGC TGCAGCAGAA GGAAGGGCTT AGCCAGGGTG GGCCTGGCG | 1773 |
| TCTGTGTTGA AGGGAAACCA AGCAGAAGCC ACTGTAATGA TATGTCACAA TAAAACCCAT | 1833 |
| GAATGAAAAA AAAAAAAAAA AAAAAAAAAA AAAAGAATTC | 1873 |

20) INFORMATION FOR SEQ ID NO:19:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 430 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

- (D) OTHER INFORMATION: /product= "mOP1-PP"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

```

Met His Val Arg Ser Leu Arg Ala Ala Ala Pro His Ser Phe Val Ala
 1           5           10           15
Leu Trp Ala Pro Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser
          20           25           30
Leu Asp Asn Glu Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser
          35           40           45
Gln Glu Arg Arg Glu Met Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu
          50           55           60
Pro His Arg Pro Arg Pro His Leu Gln Gly Lys His Asn Ser Ala Pro
          65           70           75           80
Met Phe Met Leu Asp Leu Tyr Asn Ala Met Ala Val Glu Glu Ser Gly
          85           90           95
Pro Asp Gly Gln Gly Phe Ser Tyr Pro Tyr Lys Ala Val Phe Ser Thr
          100          105          110
Gln Gly Pro Pro Leu Ala Ser Leu Gln Asp Ser His Phe Leu Thr Asp
          115          120          125
Ala Asp Met Val Met Ser Phe Val Asn Leu Val Glu His Asp Lys Glu
          130          135          140
Phe Phe His Pro Arg Tyr His His Arg Glu Phe Arg Phe Asp Leu Ser
          145          150          155          160
Lys Ile Pro Glu Gly Glu Arg Val Thr Ala Ala Glu Phe Arg Ile Tyr
          165          170          175
Lys Asp Tyr Ile Arg Glu Arg Phe Asp Asn Glu Thr Phe Gln Ile Thr
          180          185          190
Val Tyr Gln Val Leu Gln Glu His Ser Gly Arg Glu Ser Asp Leu Phe
          195          200          205

```

Leu L u Asp S r Arg Thr Il Trp Ala Ser Glu Glu Gly Trp Leu Val
 210 215 220
 Phe Asp Ile Thr Ala Thr Ser Asn His Trp Val Val Asn Pro Arg His
 225 230 235 240
 Asn Leu Gly Leu Gln Leu Ser Val Glu Thr Leu Asp Gly Gln Ser Ile
 245 250 255
 Asn Pro Lys Leu Ala Gly Leu Ile Gly Arg His Gly Pro Gln Asn Lys
 260 265 270
 Gln Pro Phe Met Val Ala Phe Phe Lys Ala Thr Glu Val His Leu Arg
 275 280 285
 Ser Ile Arg Ser Thr Gly Gly Lys Gln Arg Ser Gln Asn Arg Ser Lys
 290 295 300
 Thr Pro Lys Asn Gln Glu Ala Leu Arg Met Ala Ser Val Ala Glu Asn
 305 310 315 320
 Ser Ser Ser Asp Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr Val
 325 330 335
 Ser Phe Arg Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu Gly
 340 345 350
 Tyr Ala Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn Ser
 355 360 365
 Tyr Met Asn Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His Phe
 370 375 380
 Ile Asn Pro Asp Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln Leu
 385 390 395 400
 Asn Ala Ile Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile Leu
 405 410 415
 Lys Lys Tyr Arg Asn Met Val Val Arg Ala Cys Gly Cys His
 420 425 430

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1723 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo sapiens
- (F) TISSUE TYPE: HIPPOCAMPUS

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 490..1696
- (D) OTHER INFORMATION: /note= "hOP2 (cDNA)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

```

GGCGCCGGCA GAGCAGGAGT GGCTGGAGGA GCTGTGGTTG GAGCAGGAGG TGGCACGGCA      60
GGGCTGGAGG GCTCCCTATG AGTGGCGGAG ACGGCCAGG AGGCGCTGGA GCAACAGCTC      120
CCACACCGCA CCAAGCGGTG GCTGCAGGAG CTCGCCCATC GCCCCTGCGC TGCTCGGACC      180
GCGGCCACAG CCGGACTGGC GGGTACGGCG GCGACAGAGG CATTGGCCGA GAGTCCCACT      240
CCGCAGAGTA GCGCCGGCCT CGAGGCGGTG GCGTCCCGGT CCTCTCCGTC CAGGAGCCAG      300
GACAGGTGTC GCGCGGCGGG GCTCCAGGGA CCGCGCCTGA GGCGGGCTGC CCGCCCGTCC      360
CGCCCCGCCC CGCCGCCCCG CGCCGCGCGA GCCCAGCCTC CTGCGCGTCG GGGCGTCCCC      420
AGGCCCTGGG TCGGCGCGGG AGCCGATGCG CGCCCGCTGA GCGCCCCAGC TGAGCGCCCC      480
CGGCCTGCC ATG ACC GCG CTC CCC GGC CCG CTC TGG CTC CTG GGC CTG      528
      Met Thr Ala Leu Pro Gly Pro Leu Trp Leu Leu Gly Leu
        1             5             10

GCG CTA TGC GCG CTG GGC GGG GGC GGC CCC GGC CTG CGA CCC CCG CCC      576
Ala Leu Cys Ala Leu Gly Gly Gly Gly Pro Gly Leu Arg Pro Pro Pro
      15             20             25

GGC TGT CCC CAG CGA CGT CTG GGC GCG CGC GAG CGC CGG GAC GTG CAG      624
Gly Cys Pro Gln Arg Arg Leu Gly Ala Arg Glu Arg Arg Asp Val Gln
      30             35             40             45

CGC GAG ATC CTG GCG GTG CTC GGG CTG CCT GGG CGG CCC CGG CCC CGC      672
Arg Glu Ile Leu Ala Val Leu Gly Leu Pro Gly Arg Pro Arg Pro Arg
      50             55             60

```

| | |
|---|------|
| GCG CCA CCC GCC GCC TCC CGG CTG CCC GCG TCC GCG CCG CTC TTC ATG Ala Pro Pro Ala Ala Ser Arg Leu Pro Ala Ser Ala Pro Leu Phe Met 65 70 75 | 720 |
| CTG GAC CTG TAC CAC GCC ATG GCC GGC GAC GAC GAC GAG GAC GGC GCG Leu Asp Leu Tyr His Ala Met Ala Gly Asp Asp Asp Glu Asp Gly Ala 80 85 90 | 768 |
| CCC GCG GAG CGG CGC CTG GGC CGC GCC GAC CTG GTC ATG AGC TTC GTT Pro Ala Glu Arg Arg Leu Gly Arg Ala Asp Leu Val Met Ser Phe Val 95 100 105 | 816 |
| AAC ATG GTG GAG CGA GAC CGT GCC CTG GGC CAC CAG GAG CCC CAT TGG Asn Met Val Glu Arg Asp Arg Ala Leu Gly His Gln Glu Pro His Trp 110 115 120 125 | 864 |
| AAG GAG TTC CGC TTT GAC CTG ACC CAG ATC CCG GCT GGG GAG GCG GTC Lys Glu Phe Arg Phe Asp Leu Thr Gln Ile Pro Ala Gly Glu Ala Val 130 135 140 | 912 |
| ACA GCT GCG GAG TTC CGG ATT TAC AAG GTG CCC AGC ATC CAC CTG CTC Thr Ala Ala Glu Phe Arg Ile Tyr Lys Val Pro Ser Ile His Leu Leu 145 150 155 | 960 |
| AAC AGG ACC CTC CAC GTC AGC ATG TTC CAG GTG GTC CAG GAG CAG TCC Asn Arg Thr Leu His Val Ser Met Phe Gln Val Val Gln Glu Gln Ser 160 165 170 | 1008 |
| AAC AGG GAG TCT GAC TTG TTC TTT TTG GAT CTT CAG ACG CTC CGA GCT Asn Arg Glu Ser Asp Leu Phe Phe Leu Asp Leu Gln Thr Leu Arg Ala 175 180 185 | 1056 |
| GGA GAC GAG GGC TGG CTG GTG CTG GAT GTC ACA GCA GCC AGT GAC TGC Gly Asp Glu Gly Trp Leu Val Leu Asp Val Thr Ala Ala Ser Asp Cys 190 195 200 205 | 1104 |
| TGG TTG CTG AAG CGT CAC AAG GAC CTG GGA CTC CGC CTC TAT GTG GAG Trp Leu Leu Lys Arg His Lys Asp Leu Gly Leu Arg Leu Tyr Val Glu 210 215 220 | 1152 |
| ACT GAG GAC GGG CAC AGC GTG GAT CCT GGC CTG GCC GGC CTG CTG GGT Thr Glu Asp Gly His Ser Val Asp Pro Gly Leu Ala Gly Leu Leu Gly 225 230 235 | 1200 |
| CAA CGG GCC CCA CGC TCC CAA CAG CCT TTC GTG GTC ACT TTC TTC AGG Gln Arg Ala Pro Arg Ser Gln Gln Pro Phe Val Val Thr Phe Phe Arg 240 245 250 | 1248 |
| GCC AGT CCG AGT CCC ATC CGC ACC CCT CGG GCA GTG AGG CCA CTG AGG Ala Ser Pro Ser Pro Ile Arg Thr Pro Arg Ala Val Arg Pro Leu Arg 255 260 265 | 1296 |

| | |
|---|------|
| AGG AGG CAG CCG AAG AAA AGC AAC GAG CTG CCG CAG GCC AAC CGA CTC Arg Arg Gln Pro Lys Lys Ser Asn Glu Leu Pro Gln Ala Asn Arg Leu 270 275 280 285 | 1344 |
| CCA GGG ATC TTT GAT GAC GTC CAC GGC TCC CAC GGC CGG CAG GTC TGC Pro Gly Ile Phe Asp Asp Val His Gly Ser His Gly Arg Gln Val Cys 290 295 300 | 1392 |
| CGT CGG CAC GAG CTC TAC GTC AGC TTC CAG GAC CTC GGC TGG CTG GAC Arg Arg His Glu Leu Tyr Val Ser Phe Gln Asp Leu Gly Trp Leu Asp 305 310 315 | 1440 |
| TGG GTC ATC GCT CCC CAA GGC TAC TCG GCC TAT TAC TGT GAG GGG GAG Trp Val Ile Ala Pro Gln Gly Tyr Ser Ala Tyr Tyr Cys Glu Gly Glu 320 325 330 | 1488 |
| TGC TCC TTC CCA CTG GAC TCC TGC ATG AAT GCC ACC AAC CAC GCC ATC Cys Ser Phe Pro Leu Asp Ser Cys Met Asn Ala Thr Asn His Ala Ile 335 340 345 | 1536 |
| CTG CAG TCC CTG GTG CAC CTG ATG AAG CCA AAC GCA GTC CCC AAG GCG Leu Gln Ser Leu Val His Leu Met Lys Pro Asn Ala Val Pro Lys Ala 350 355 360 365 | 1584 |
| TGC TGT GCA CCC ACC AAG CTG AGC GCC ACC TCT GTG CTC TAC TAT GAC Cys Cys Ala Pro Thr Lys Leu Ser Ala Thr Ser Val Leu Tyr Tyr Asp 370 375 380 | 1632 |
| AGC AGC AAC AAC GTC ATC CTG CGC AAA CAC CGC AAC ATG GTG GTC AAG Ser Ser Asn Asn Val Ile Leu Arg Lys His Arg Asn Met Val Val Lys 385 390 395 | 1680 |
| GCC TGC GGC TGC CAC T GAGTCAGCCC GCCCAGCCCT ACTGCAG Ala Cys Gly Cys His 400 | 1723 |

(2) INFORMATION FOR SEQ ID NO:21:

(i)SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 402 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii)MOLECULE TYPE: protein

(ix)FEATURE:

- (A)OTHER INFORMATION: /product= "hOP2-PP"

(xi)SEQUENCE DESCRIPTION: SEQ ID NO:21:

```

Met Thr Ala Leu Pro Gly Pro Leu Trp Leu Leu Gly Leu Ala Leu Cys
 1              5              10              15

Ala Leu Gly Gly Gly Gly Pro Gly Leu Arg Pro Pro Pro Gly Cys Pro
      20              25              30

Gln Arg Arg Leu Gly Ala Arg Glu Arg Arg Asp Val Gln Arg Glu Ile
      35              40              45

Leu Ala Val Leu Gly Leu Pro Gly Arg Pro Arg Pro Arg Ala Pro Pro
      50              55              60

Ala Ala Ser Arg Leu Pro Ala Ser Ala Pro Leu Phe Met Leu Asp Leu
      65              70              75              80

Tyr His Ala Met Ala Gly Asp Asp Asp Glu Asp Gly Ala Pro Ala Glu
      85              90              95

Arg Arg Leu Gly Arg Ala Asp Leu Val Met Ser Phe Val Asn Met Val
      100             105             110

Glu Arg Asp Arg Ala Leu Gly His Gln Glu Pro His Trp Lys Glu Phe
      115             120             125

Arg Phe Asp Leu Thr Gln Ile Pro Ala Gly Glu Ala Val Thr Ala Ala
      130             135             140

Glu Phe Arg Ile Tyr Lys Val Pro Ser Ile His Leu Leu Asn Arg Thr
      145             150             155             160

Leu His Val Ser Met Phe Gln Val Val Gln Glu Gln Ser Asn Arg Glu
      165             170             175

Ser Asp Leu Phe Phe Leu Asp Leu Gln Thr Leu Arg Ala Gly Asp Glu
      180             185             190

Gly Trp Leu Val Leu Asp Val Thr Ala Ala Ser Asp Cys Trp Leu Leu
      195             200             205

```


Lys Arg His Lys Asp Leu Gly Leu Arg Leu Tyr Val Glu Thr Glu Asp
 210 215 220
 Gly His Ser Val Asp Pro Gly Leu Ala Gly Leu Leu Gly Gln Arg Ala
 225 230 235 240
 Pro Arg Ser Gln Gln Pro Phe Val Val Thr Phe Phe Arg Ala Ser Pro
 245 250 255
 Ser Pro Ile Arg Thr Pro Arg Ala Val Arg Pro Leu Arg Arg Arg Gln
 260 265 270
 Pro Lys Lys Ser Asn Glu Leu Pro Gln Ala Asn Arg Leu Pro Gly Ile
 275 280 285
 Phe Asp Asp Val His Gly Ser His Gly Arg Gln Val Cys Arg Arg His
 290 295 300
 Glu Leu Tyr Val Ser Phe Gln Asp Leu Gly Trp Leu Asp Trp Val Ile
 305 310 315 320
 Ala Pro Gln Gly Tyr Ser Ala Tyr Tyr Cys Glu Gly Glu Cys Ser Phe
 325 330 335
 Pro Leu Asp Ser Cys Met Asn Ala Thr Asn His Ala Ile Leu Gln Ser
 340 345 350
 Leu Val His Leu Met Lys Pro Asn Ala Val Pro Lys Ala Cys Cys Ala
 355 360 365
 Pro Thr Lys Leu Ser Ala Thr Ser Val Leu Tyr Tyr Asp Ser Ser Asn
 370 375 380
 Asn Val Ile Leu Arg Lys His Arg Asn Met Val Val Lys Ala Cys Gly
 385 390 395 400
 Cys His

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1926 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: MURIDAE
- (F) TISSUE TYPE: EMBRYO

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 93..1289
- (D) OTHER INFORMATION: /note= "mOP2 cDNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

```

GCCAGGCACA GGTGCGCCGT CTGGTCCTCC CCGTCTGGCG TCAGCCGAGC      50
CCGACCAGCT ACCAGTGGAT GCGCGCCGGC TGAAAGTCCG AG ATG GCT ATG CGT      104
                                     Met Ala Met Arg
                                     1

CCC GGG CCA CTC TGG CTA TTG GGC CTT GCT CTG TGC GCG CTG GGA GGC      152
Pro Gly Pro Leu Trp Leu Leu Gly Leu Ala Leu Cys Ala Leu Gly Gly
  5                10                15                20

GGC CAC GGT CCG CGT CCC CCG CAC ACC TGT CCC CAG CGT CGC CTG GGA      200
Gly His Gly Pro Arg Pro Pro His Thr Cys Pro Gln Arg Arg Leu Gly
                25                30                35

GCG CGC GAG CGC CGC GAC ATG CAG CGT GAA ATC CTG GCG GTG CTC GGG      248
Ala Arg Glu Arg Arg Asp Met Gln Arg Glu Ile Leu Ala Val Leu Gly
                40                45                50

CTA CCG GGA CGG CCC CGA CCC CGT GCA CAA CCC GCG GCT GCC CGG CAG      296
Leu Pro Gly Arg Pro Arg Pro Arg Ala Gln Pro Ala Ala Ala Arg Gln
                55                60                65

CCA GCG TCC GCG CCC CTC TTC ATG TTG GAC CTA TAC CAC GCC ATG ACC      344
Pro Ala Ser Ala Pro Leu Phe Met Leu Asp Leu Tyr His Ala Met Thr
                70                75                80

GAT GAC GAC GAC GGC GGG CCA CCA CAG GCT CAC TTA GGC CGT GCC GAC      392
Asp Asp Asp Asp Gly Gly Pro Pro Gln Ala His Leu Gly Arg Ala Asp
  85                90                95                100

```

| | |
|---|------|
| CTG GTC ATG AGC TTC GTC AAC ATG GTG GAA CGC GAC CGT ACC CTG GGC Leu Val Met Ser Phe Val Asn Met Val Glu Arg Asp Arg Thr Leu Gly 105 110 115 | 440 |
| TAC CAG GAG CCA CAC TGG AAG GAA TTC CAC TTT GAC CTA ACC CAG ATC Tyr Gln Glu Pro His Trp Lys Glu Phe His Phe Asp Leu Thr Gln Ile 120 125 130 | 488 |
| CCT GCT GGG GAG GCT GTC ACA GCT GCT GAG TTC CGG ATC TAC AAA GAA Pro Ala Gly Glu Ala Val Thr Ala Ala Glu Phe Arg Ile Tyr Lys Glu 135 140 145 | 536 |
| CCC AGC ACC CAC CCG CTC AAC ACA ACC CTC CAC ATC AGC ATG TTC GAA Pro Ser Thr His Pro Leu Asn Thr Thr Leu His Ile Ser Met Phe Glu 150 155 160 | 584 |
| GTG GTC CAA GAG CAC TCC AAC AGG GAG TCT GAC TTG TTC TTT TTG GAT Val Val Gln Glu His Ser Asn Arg Glu Ser Asp Leu Phe Phe Leu Asp 165 170 175 180 | 632 |
| CTT CAG ACG CTC CGA TCT GGG GAC GAG GGC TGG CTG GTG CTG GAC ATC Leu Gln Thr Leu Arg Ser Gly Asp Glu Gly Trp Leu Val Leu Asp Ile 185 190 195 | 680 |
| ACA GCA GCC AGT GAC CGA TGG CTG CTG AAC CAT CAC AAG GAC CTG GGA Thr Ala Ala Ser Asp Arg Trp Leu Leu Asn His His Lys Asp Leu Gly 200 205 210 | 728 |
| CTC CGC CTC TAT GTG GAA ACC GCG GAT GGG CAC AGC ATG GAT CCT GGC Leu Arg Leu Tyr Val Glu Thr Ala Asp Gly His Ser Met Asp Pro Gly 215 220 225 | 776 |
| CTG GCT GGT CTG CTT GGA CGA CAA GCA CCA CGC TCC AGA CAG CCT TTC Leu Ala Gly Leu Leu Gly Arg Gln Ala Pro Arg Ser Arg Gln Pro Phe 230 235 240 | 824 |
| ATG GTA ACC TTC TTC AGG GCC AGC CAG AGT CCT GTG CGG GCC CCT CGG Met Val Thr Phe Phe Arg Ala Ser Gln Ser Pro Val Arg Ala Pro Arg 245 250 255 260 | 872 |
| GCA GCG AGA CCA CTG AAG AGG AGG CAG CCA AAG AAA ACG AAC GAG CTT Ala Ala Arg Pro Leu Lys Arg Arg Gln Pro Lys Lys Thr Asn Glu Leu 265 270 275 | 920 |
| CCG CAC CCC AAC AAA CTC CCA GGG ATC TTT GAT GAT GGC CAC GGT TCC Pro His Pro Asn Lys Leu Pro Gly Ile Phe Asp Asp Gly His Gly Ser 280 285 290 | 968 |
| CGC GGC AGA GAG GTT TGC CGC AGG CAT GAG CTC TAC GTC AGC TTC CGT Arg Gly Arg Glu Val Cys Arg Arg His Glu Leu Tyr Val Ser Phe Arg 295 300 305 | 1016 |

| | |
|---|------|
| GAC CTT GGC TGG CTG GAC TGG GTC ATC GCC CCC CAG GGC TAC TCT GCC Asp Leu Gly Trp Leu Asp Trp Val Ile Ala Pro Gln Gly Tyr Ser Ala 310 315 320 | 1064 |
| TAT TAC TGT GAG GGG GAG TGT GCT TTC CCA CTG GAC TCC TGT ATG AAC Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asp Ser Cys Met Asn 325 330 335 340 | 1112 |
| GCC ACC AAC CAT GCC ATC TTG CAG TCT CTG GTG CAC CTG ATG AAG CCA Ala Thr Asn His Ala Ile Leu Gln Ser Leu Val His Leu Met Lys Pro 345 350 355 | 1160 |
| GAT GTT GTC CCC AAG GCA TGC TGT GCA CCC ACC AAA CTG AGT GCC ACC Asp Val Val Pro Lys Ala Cys Cys Ala Pro Thr Lys Leu Ser Ala Thr 360 365 370 | 1208 |
| TCT GTG CTG TAC TAT GAC AGC AGC AAC AAT GTC ATC CTG CGT AAA CAC Ser Val Leu Tyr Tyr Asp Ser Ser Asn Asn Val Ile Leu Arg Lys His 375 380 385 | 1256 |
| CGT AAC ATG GTG GTC AAG GCC TGT GGC TGC CAC TGAGGCCCG CCCAGCATCC Arg Asn Met Val Val Lys Ala Cys Gly Cys His 390 395 | 1309 |
| TGCTTCTACT ACCTTACCAT CTGGCCGGGC CCCTCTCCAG AGGCAGAAAC CCTTCTATGT | 1369 |
| TATCATAGCT CAGACAGGGG CAATGGGAGG CCCTTCACTT CCCCTGGCCA CTTCTTGCTA | 1429 |
| AAATTCTGGT CTTTCCCACT TCCTCTGTCC TTCATGGGGT TTCGGGGCTA TCACCCCGCC | 1489 |
| CTCTCCATCC TCCTACCCCA AGCATAGACT GAATGCACAC AGCATCCCAG AGCTATGCTA | 1549 |
| ACTGAGAGGT CTGGGGTCAG CACTGAAGGC CCACATGAGG AAGACTGATC CTTGGCCATC | 1609 |
| CTCAGCCAC AATGGCAAAT TCTGGATGGT CTAAGAAGGC CGTGGAATTC TAACTAGAT | 1669 |
| GATCTGGGCT CTCTGCACCA TTCATTGTGG CAGTTGGGAC ATTTTATAGGT ATAACAGACA | 1729 |
| CATACACTTA GATCAATGCA TCGCTGTACT CCTTGAAATC AGAGCTAGCT TGTTAGAAAA | 1789 |
| AGAATCAGAG CCAGGTATAG CGGTGCATGT CATTAAATCCC AGCGCTAAAG AGACAGAGAC | 1849 |
| AGGAGAATCT CTGTGAGTTC AAGGCCACAT AGAAAGAGCC TGTCTCGGGA GCAGGAAAAA | 1909 |
| AAAAAAAAAC GGAATTC | 1926 |

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 399 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

- (D) OTHER INFORMATION: /product= "mOP2-PP"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

```

Met Ala Met Arg Pro Gly Pro Leu Trp Leu Leu Gly Leu Ala Leu Cys
 1           5           10           15
Ala Leu Gly Gly Gly His Gly Pro Arg Pro Pro His Thr Cys Pro Gln
          20           25           30
Arg Arg Leu Gly Ala Arg Glu Arg Arg Asp Met Gln Arg Glu Ile Leu Ala
          35           40           45
Val Leu Gly Leu Pro Gly Arg Pro Arg Pro Arg Ala Gln Pro Ala Ala
 50           55           60           65
Ala Arg Gln Pro Ala Ser Ala Pro Leu Phe Met Leu Asp Leu Tyr His Ala
          70           75           80
Met Thr Asp Asp Asp Asp Gly Gly Pro Pro Gln Ala His Leu Gly Arg
          85           90           95
Ala Asp Leu Val Met Ser Phe Val Asn Met Val Glu Arg Asp Arg Thr
100           105           110
Leu Gly Tyr Gln Glu Pro His Trp Lys Glu Phe His Phe Asp Leu Thr
115           120           125           130
Gln Ile Pro Ala Gly Glu Ala Val Thr Ala Ala Glu Phe Arg Ile Tyr
          135           140           145
Lys Glu Pro Ser Thr His Pro Leu Asn Thr Thr Leu His Ile Ser Met
          150           155           160
Phe Glu Val Val Gln Glu His Ser Asn Arg Glu Ser Asp Leu Phe Phe
          165           170           175
Leu Asp Leu Gln Thr Leu Arg Ser Gly Asp Glu Gly Trp Leu Val Leu
180           185           190
Asp Ile Thr Ala Ala Ser Asp Arg Trp Leu Leu Asn His His Lys Asp
195           200           205           210

```

Leu Gly Leu Arg Leu Tyr Val Glu Thr Ala Asp Gly His Ser Met Asp
 215 220 225
 Pro Gly Leu Ala Gly Leu Leu Gly Arg Gln Ala Pro Arg Ser Arg Gln
 230 235 240
 Pro Phe Met Val Thr Phe Phe Arg Ala Ser Gln Ser Pro Val Arg Ala
 245 250 255
 Pro Arg Ala Ala Arg Pro Leu Lys Arg Arg Gln Pro Lys Lys Thr Asn
 260 265 270
 Glu Leu Pro His Pro Asn Lys Leu Pro Gly Ile Phe Asp Asp Gly His
 275 280 285 290
 Gly Ser Arg Gly Arg Glu Val Cys Arg Arg His Glu Leu Tyr Val Ser
 295 300 305
 Phe Arg Asp Leu Gly Trp Leu Asp Trp Val Ile Ala Pro Gln Gly Tyr
 310 315 320
 Ser Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asp Ser Cys
 325 330 335
 Met Asn Ala Thr Asn His Ala Ile Leu Gln Ser Leu Val His Leu Met
 340 345 350
 Lys Pro Asp Val Val Pro Lys Ala Cys Cys Ala Pro Thr Lys Leu Ser
 355 360 365 370
 Ala Thr Ser Val Leu Tyr Tyr Asp Ser Ser Asn Asn Val Ile Leu Arg
 375 380 385
 Lys His Arg Asn Met Val Val Lys Ala Cys Gly Cys His
 390 395

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1368 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..1368
- (D) OTHER INFORMATION: /STANDARD NAME="60A"

(x) PUBLICATION INFORMATION:

- (A) AUTHORS: WHARTON, KRISTI A.; THOMSEN, GERALD H.; GELBERT, WILLIAM M.
- (B) TITLE: DROSOPHILA 60A GENE...
- (C) JOURNAL: PROC. NAT'L ACAD. SCI. USA
- (D) VOLUME: 88
- (E) RELEVANT RESIDUES IN SEQ ID NO:3: FROM 1 TO 1368
- (F) PAGES: 9214-9218
- (G) DATE: OCT - 1991

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

| | |
|---|-----|
| ATG TCG GGA CTG CGA AAC ACC TCG GAG GCC GTT GCA GTG CTC GCC TCC | 48 |
| Met Ser Gly Leu Arg Asn Thr Ser Glu Ala Val Ala Val Leu Ala Ser | |
| 1 5 10 15 | |
| CTG GGA CTC GGA ATG GTT CTG CTC ATG TTC GTG GCG ACC ACG CCG CCG | 96 |
| Leu Gly Leu Gly Met Val Leu Leu Met Phe Val Ala Thr Thr Pro Pro | |
| 20 25 30 | |
| GCC GTT GAG GCC ACC CAG TCG GGG ATT TAC ATA GAC AAC GGC AAG GAC | 144 |
| Ala Val Glu Ala Thr Gln Ser Gly Ile Tyr Ile Asp Asn Gly Lys Asp | |
| 35 40 45 | |
| CAG ACG ATC ATG CAC AGA GTG CTG AGC GAG GAC GAC AAG CTG GAC GTC | 192 |
| Gln Thr Ile Met His Arg Val Leu Ser Glu Asp Asp Lys Leu Asp Val | |
| 50 55 60 | |
| TCG TAC GAG ATC CTC GAG TTC CTG GGC ATC GCC GAA CGG CCG ACG CAC | 240 |
| Ser Tyr Glu Ile Leu Glu Phe Leu Gly Ile Ala Glu Arg Pro Thr His | |
| 65 70 75 80 | |
| CTG AGC AGC CAC CAG TTG TCG CTG AGG AAG TCG GCT CCC AAG TTC CTG | 288 |
| Leu Ser Ser His Gln Leu Ser Leu Arg Lys Ser Ala Pro Lys Phe Leu | |
| 85 90 95 | |

| | |
|---|-----|
| CTG GAC GTC TAC CAC CGC ATC ACG GCG GAG GAG GGT CTC AGC GAT CAG Leu Asp Val Tyr His Arg Ile Thr Ala Glu Glu Gly Leu Ser Asp Gln 100 105 110 | 336 |
| GAT GAG GAC GAC GAC TAC GAA CGC GGC CAT CGG TCC AGG AGG AGC GCC Asp Glu Asp Asp Asp Tyr Glu Arg Gly His Arg Ser Arg Arg Ser Ala 115 120 125 | 384 |
| GAC CTC GAG GAG GAT GAG GGC GAG CAG CAG AAG AAC TTC ATC ACC GAC Asp Leu Glu Glu Asp Glu Gly Glu Gln Gln Lys Asn Phe Ile Thr Asp 130 135 140 | 432 |
| CTG GAC AAG CGG GCC ATC GAC GAG AGC GAC ATC ATC ATG ACC TTC CTG Leu Asp Lys Arg Ala Ile Asp Glu Ser Asp Ile Ile Met Thr Phe Leu 145 150 155 160 | 480 |
| AAC AAG CGC CAC CAC AAT GTG GAC GAA CTG CGT CAC GAG CAC GGC CGT Asn Lys Arg His His Asn Val Asp Glu Leu Arg His Glu His Gly Arg 165 170 175 | 528 |
| CGC CTG TGG TTC GAC GTC TCC AAC GTG CCC AAC GAC AAC TAC CTG GTG Arg Leu Trp Phe Asp Val Ser Asn Val Pro Asn Asp Asn Tyr Leu Val 180 185 190 | 576 |
| ATG GCC GAG CTG CGC ATC TAT CAG AAC GCC AAC GAG GGC AAG TGG CTG Met Ala Glu Leu Arg Ile Tyr Gln Asn Ala Asn Glu Gly Lys Trp Leu 195 200 205 | 624 |
| ACC GCC AAC AGG GAG TTC ACC ATC ACG GTA TAC GCC ATT GGC ACC GGC Thr Ala Asn Arg Glu Phe Thr Ile Thr Val Tyr Ala Ile Gly Thr Gly 210 215 220 | 672 |
| ACG CTG GGC CAG CAC ACC ATG GAG CCG CTG TCC TCG GTG AAC ACC ACC Thr Leu Gly Gln His Thr Met Glu Pro Leu Ser Ser Val Asn Thr Thr 225 230 235 240 | 720 |
| GGG GAC TAC GTG GGC TGG TTG GAG CTC AAC GTG ACC GAG GGC CTG CAC Gly Asp Tyr Val Gly Trp Leu Glu Leu Asn Val Thr Glu Gly Leu His 245 250 255 | 768 |
| GAG TGG CTG GTC AAG TCG AAG GAC AAT CAT GGC ATC TAC ATT GGA GCA Glu Trp Leu Val Lys Ser Lys Asp Asn His Gly Ile Tyr Ile Gly Ala 260 265 270 | 816 |
| CAC GCT GTC AAC CGA CCC GAC CGC GAG GTG AAG CTG GAC GAC ATT GGA His Ala Val Asn Arg Pro Asp Arg Glu Val Lys Leu Asp Asp Ile Gly 275 280 285 | 864 |
| CTG ATC CAC CGC AAG GTG GAC GAC GAG TTC CAG CCC TTC ATG ATC GGC Leu Ile His Arg Lys Val Asp Asp Glu Phe Gln Pro Phe Met Ile Gly 290 295 300 | 912 |

[illegible]

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 455 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Met Ser Gly Leu Arg Asn Thr Ser Glu Ala Val Ala Val Leu Ala Ser
1 5 10 15
Leu Gly Leu Gly Met Val Leu Leu Met Phe Val Ala Thr Thr Pro Pro
20 25 30
Ala Val Glu Ala Thr Gln Ser Gly Ile Tyr Ile Asp Asn Gly Lys Asp
35 40 45
Gln Thr Ile Met His Arg Val Leu Ser Glu Asp Asp Lys Leu Asp Val
50 55 60
Ser Tyr Glu Ile Leu Glu Phe Leu Gly Ile Ala Glu Arg Pro Thr His
65 70 75 80
Leu Ser Ser His Gln Leu Ser Leu Arg Lys Ser Ala Pro Lys Phe Leu
85 90 95
Leu Asp Val Tyr His Arg Ile Thr Ala Glu Glu Gly Leu Ser Asp Gln
100 105 110
Asp Glu Asp Asp Asp Tyr Glu Arg Gly His Arg Ser Arg Arg Ser Ala
115 120 125
Asp Leu Glu Glu Asp Glu Gly Glu Gln Gln Lys Asn Phe Ile Thr Asp
130 135 140
Leu Asp Lys Arg Ala Ile Asp Glu Ser Asp Ile Ile Met Thr Phe Leu
145 150 155 160
Asn Lys Arg His His Asn Val Asp Glu Leu Arg His Glu His Gly Arg
165 170 175
Arg Leu Trp Phe Asp Val Ser Asn Val Pro Asn Asp Asn Tyr Leu Val
180 185 190
Met Ala Glu Leu Arg Ile Tyr Gln Asn Ala Asn Glu Gly Lys Trp Leu
195 200 205
Thr Ala Asn Arg Glu Phe Thr Ile Thr Val Tyr Ala Ile Gly Thr Gly
210 215 220

Thr Leu Gly Gln His Thr M t Glu Pro Leu Ser Ser Val Asn Thr Thr
 225 230 235 240
 Gly Asp Tyr Val Gly Trp Leu Glu Leu Asn Val Thr Glu Gly Leu His
 245 250 255
 Glu Trp Leu Val Lys Ser Lys Asp Asn His Gly Ile Tyr Ile Gly Ala
 260 265 270
 His Ala Val Asn Arg Pro Asp Arg Glu Val Lys Leu Asp Asp Ile Gly
 275 280 285
 Leu Ile His Arg Lys Val Asp Asp Glu Phe Gln Pro Phe Met Ile Gly
 290 295 300
 Phe Phe Arg Gly Pro Glu Leu Ile Lys Ala Thr Ala His Ser Ser His
 305 310 315 320
 His Arg Ser Lys Arg Ser Ala Ser His Pro Arg Lys Arg Lys Lys Ser
 325 330 335
 Val Ser Pro Asn Asn Val Pro Leu Leu Glu Pro Met Glu Ser Thr Arg
 340 345 350
 Ser Cys Gln Met Gln Thr Leu Tyr Ile Asp Phe Lys Asp Leu Gly Trp
 355 360 365
 His Asp Trp Ile Ile Ala Pro Glu Gly Tyr Gly Ala Phe Tyr Cys Ser
 370 375 380
 Gly Glu Cys Asn Phe Pro Leu Asn Ala His Met Asn Ala Thr Asn His
 385 390 395 400
 Ala Ile Val Gln Thr Leu Val His Leu Leu Glu Pro Lys Lys Val Pro
 405 410 415
 Lys Pro Cys Cys Ala Pro Thr Arg Leu Gly Ala Leu Pro Val Leu Tyr
 420 425 430
 His Leu Asn Asp Glu Asn Val Asn Leu Lys Lys Tyr Arg Asn Met Ile
 435 440 445
 Val Lys Ser Cys Gly Cys His
 450 455

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) ORIGINAL SOURCE:

- (A) ORGANISM: Homo Sapiens

(ix) FEATURE:

- (A) NAME/KEY: Protein
- (B) LOCATION: 1..102
- (D) OTHER INFORMATION: /note="BMP3"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 104 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

- (A) NAME/KEY: Protein
- (B) LOCATION: 1..104
- (D) OTHER INFORMATION: /note="BMP3"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

```

Cys Ala Arg Arg Tyr Leu Lys Val Asp Phe Ala Asp Ile Gly Trp Ser
1      5      10
Glu Trp Ile Ile Ser Pro Lys Ser Phe Asp Ala Tyr Try Cys Ser Gly
20     25     30
Ala Cys Gln Phe Pro Met Pro Lys Ser Leu Lys Pro Ser Asn His Ala
35     40     45
Thr Ile Gln Ser Ile Val Ala Arg Ala Val Gly Val Val Pro Gly Ile
50     55     60
Pro Glu Pro Cys Cys Val Pro Glu Lys Met Ser Ser Leu Ser Ile Leu
65     70     75     80
Phe Phe Asp Glu Asn Lys Asn Val Val Leu Lys Val Tyr Pro Asn Met
85     90     95
Thr Val Glu Ser Cys Ala Cys Arg
100

```

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 102 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: HOMO SAPIENS

(ix) FEATURE:

- (A) NAME/KEY: Protein
- (B) LOCATION: 1..102
- (D) OTHER INFORMATION: /note= "BMP5"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

```

Cys Lys Lys His Glu Leu Tyr Val Ser Phe Arg Asp Leu Gly Trp Gln
1          5          10          15
Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala Ala Phe Tyr Cys Asp Gly
20          25          30
Glu Cys Ser Phe Pro Leu Asn Ala His Met Asn Ala Thr Asn His Ala
35          40          45
Ile Val Gln Thr Leu Val His Leu Met Phe Pro Asp His Val Pro Lys
50          55          60
Pro Cys Cys Ala Pro Thr Lys Leu Asn Ala Ile Ser Val Leu Tyr Phe
65          70          75          80
Asp Asp Ser Ser Asn Val Ile Leu Lys Lys Tyr Arg Asn Met Val Val
85          90          95
Arg Ser Cys Gly Cys His
100

```

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 102 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: HOMO SAPIENS

(ix) FEATURE:

- (A) NAME/KEY: Protein
- (B) LOCATION: 1..102
- (D) OTHER INFORMATION: /note= "BMP6"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Cys Arg Lys His Glu Leu Tyr Val Ser Phe Gln Asp Leu Gly Trp Gln
1 5 10 15
Asp Trp Ile Ile Ala Pro Lys Gly Tyr Ala Ala Asn Tyr Cys Asp Gly
20 25 30
Glu Cys Ser Phe Pro Leu Asn Ala His Met Asn Ala Thr Asn His Ala
35 40 45
Ile Val Gln Thr Leu Val His Leu Met Asn Pro Glu Tyr Val Pro Lys
50 55 60
Pro Cys Cys Ala Pro Thr Lys Leu Asn Ala Ile Ser Val Leu Tyr Phe
65 70 75 80
Asp Asp Asn Ser Asn Val Ile Leu Lys Lys Tyr Arg Trp Met Val Val
85 90 95
Arg Ala Cys Gly Cys His
100

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 102 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

- (A) NAME/KEY: Protein
- (B) LOCATION: 1..102
- (D) OTHER INFORMATION: /label= OPX
/note= "WHEREIN XAA AT EACH POS'N IS INDEPENDENTLY
SELECTED FROM THE RESIDUES OCCURRING AT THE
CORRESPONDING POS'N IN THE C-TERMINAL SEQUENCE OF MOUSE
OR HUMAN OP1 OR OP2 (SEE SEQ. ID NOS. 5,6,7 and 8 or
16,18,20 and 22.)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

```

Cys Xaa Xaa His Glu Leu Tyr Val Xaa Phe Xaa Asp Leu Gly Trp Xaa
1           5           10           15
Asp Trp Xaa Ile Ala Pro Xaa Gly Tyr Xaa Ala Tyr Tyr Cys Glu Gly
20           25           30
Glu Cys Xaa Phe Pro Leu Xaa Ser Xaa Met Asn Ala Thr Asn His Ala
35           40           45
Ile Xaa Gln Xaa Leu Val His Xaa Xaa Xaa Pro Xaa Xaa Val Pro Lys
50           55           60
Xaa Cys Cys Ala Pro Thr Xaa Leu Xaa Ala Xaa Ser Val Leu Tyr Xaa
65           70           75           80
Asp Xaa Ser Xaa Asn Val Xaa Leu Xaa Lys Xaa Arg Asn Met Val Val
85           90           95
Xaa Ala Cys Gly Cys His
100

```

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 97 amino acids
- (B) TYPE: amino acids
- (C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

- (A) NAME: Generic Sequence 5
- (D) OTHER INFORMATION: wherein each Xaa is independently selected from a group of one or more specified amino acids as defined in the specification.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

```

Leu Xaa Xaa Xaa Phe
      1           5
Xaa Xaa Xaa Gly Trp Xaa Xaa Trp Xaa
      10
Xaa Xaa Pro Xaa Xaa Xaa Xaa Ala
      15           20
Xaa Tyr Cys Xaa Gly Xaa Cys Xaa
      25           30
Xaa Pro Xaa Xaa Xaa Xaa Xaa
      35
Xaa Xaa Xaa Asn His Ala Xaa Xaa
      40           45
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
      50
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
      55           60
Cys Xaa Pro Xaa Xaa Xaa Xaa Xaa
      65
Xaa Xaa Xaa Leu Xaa Xaa Xaa
      70           75
Xaa Xaa Xaa Xaa Val Xaa Leu Xaa
      80
Xaa Xaa Xaa Xaa Met Xaa Val Xaa
      85           90
Xaa Cys Xaa Cys Xaa
      95

```


(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 102 amino acids

(B) TYPE: amino acids

(C) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

(A) NAME: Generic Sequence 6

(D) OTHER INFORMATION: wherein each Xaa is independently selected from a group of one or more specified amino acids as defined in the specification.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Cys Xaa Xaa Xaa Xaa Leu Xaa Xaa Xaa Phe

1 5 10

Xaa Xaa Xaa Gly Trp Xaa Xaa Trp Xaa

15

Xaa Xaa Pro Xaa Xaa Xaa Xaa Ala

20 25

Xaa Tyr Cys Xaa Gly Xaa Cys Xaa

30 35

Xaa Pro Xaa Xaa Xaa Xaa Xaa

40

Xaa Xaa Xaa Asn His Ala Xaa Xaa

45 50

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa

55

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys

60 65

Cys Xaa Pro Xaa Xaa Xaa Xaa Xaa

70

Xaa Xaa Xaa Leu Xaa Xaa Xaa

75 80

Xaa Xaa Xaa Xaa Val Xaa Leu Xaa

85

Xaa Xaa Xaa Xaa Met Xaa Val Xaa

90 95

Xaa Cys Xaa Cys Xaa

100

(2) INFORMATION FOR SEQ ID NO:32:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1238 base pairs, 372 amino acids
 (B) TYPE: nucleic acid, amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (iii) ORIGINAL SOURCE:
 (A) ORGANISM: human
 (F) TISSUE TYPE: BRAIN
- (iv) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION:
 (D) OTHER INFORMATION:
 /product= "GDF-1"
 /note= "GDF-1 CDNA"
- (x) PUBLICATION INFORMATION:
 (A) AUTHORS: Lee, Se-Jin
 (B) TITLE: Expression of Growth/Differentiation Factor 1
 (C) JOURNAL: Proc. Nat'l Acad. Sci.
 (D) VOLUME: 88
 (E) RELEVANT RESIDUES: 1-1238
 (F) PAGES: 4250-4254
 (G) DATE: May-1991
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

```

GGGGACACCG GCCCGCCCT CAGCCCACTG GTCCCGGGCC GCCGCGGACC CTGCGCACTC      60
TCTGGTCATC GCCTGGGAGG AAG ATG CCA CCG CCG CAG CAA GGT CCC TGC GGC      113
           Met Pro Pro Pro Gln Gln Gly Pro Cys Gly
           1               5               10

CAC CAC CTC CTC CTC CTC CTG GCC CTG CTG CTG CCC TCG CTG CCC      158
His His Leu Leu Leu Leu Leu Ala Leu Leu Leu Pro Ser Leu Pro
           15               20               25

CTG ACC CGC GCC CCC GTG CCC CCA GGC CCA GCC GCC GCC CTG CTC      203
Leu Thr Arg Ala Pro Val Pro Pro Gly Pro Ala Ala Ala Leu Leu
           30               35               40

CAG GCT CTA GGA CTG CGC GAT GAG CCC CAG GGT GCC CCC AGG CTC      248
Gln Ala Leu Gly Leu Arg Asp Glu Pro Gln Gly Ala Pro Arg Leu
           45               50               55

```

| | |
|---|-----|
| CGG CCG GTT CCC CCG GTC ATG TGG CGC CTG TTT CGA CGC CGG GAC | 293 |
| Arg Pro Val Pro Pro Val Met Trp Arg Leu Phe Arg Arg Arg Asp | |
| 60 65 70 | |
| CCC CAG GAG ACC AGG TCT GGC TCG CGG CGG ACG TCC CCA GGG GTC | 338 |
| Pro Gln Glu Thr Arg Ser Gly Ser Arg Arg Thr Ser Pro Gly Val | |
| 75 80 85 | |
| ACC CTG CAA CCG TGC CAC GTG GAG GAG CTG GGG GTC GCC GGA AAC | 383 |
| Thr Leu Gln Pro Cyc His Val Glu Glu Leu Gly Val Ala Gly Asn | |
| 90 95 100 | |
| ATC GTG CGC CAC ATC CCG GAC CGC GGT GCG CCC ACC CGG GCC TCG | 428 |
| Ile Val Arg His Ile Pro Asp Arg Gly Ala Pro Thr Arg Ala Ser | |
| 105 110 115 | |
| GAG CCT GTC TCG GCC GCG GGG CAT TGC CCT GAG TGG ACA GTC GTC | 473 |
| Glu Pro Val Ser Ala Ala Gly His Cys Pro Glu Trp Thr Val Val | |
| 120 125 130 | |
| TTC GAC CTG TCG GCT GTG GAA CCC GCT GAG CGC CCG AGC CGG GCC | 518 |
| Phe Asp Leu Ser Ala Val Glu Pro Ala Glu Arg Pro Ser Arg Ala | |
| 135 140 145 | |
| CGC CTG GAG CTG CGT TTC GCG GCG GCG GCG GCG GCA GCC CCG GAG | 563 |
| Arg Leu Glu Leu Arg Phe Ala Ala Ala Ala Ala Ala Ala Pro Glu | |
| 150 155 160 | |
| GGC GGC TGG GAG CTG AGC GTG GCG CAA GCG GGC CAG GGC GCG GGC | 608 |
| Gly Gly Trp Glu Leu Ser Val Ala Gln Ala Gly Gln Gly Ala Gly | |
| 165 170 175 | |
| GCG GAC CCC GGG CCG GTG CTG CTC CGC CAG TTG GTG CCC GCC CTG | 653 |
| Ala Asp Pro Gly Pro Val Leu Leu Arg Gln Leu Val Pro Ala Leu | |
| 180 185 190 | |
| GGG CCG CCA GTG CGC GCG GAG CTG CTG GGC GCC GCT TGG GCT CGC | 698 |
| Gly Pro Pro Val Arg Ala Glu Leu Leu Gly Ala Ala Trp Ala Arg | |
| 195 200 205 | |
| AAC GCC TCA TGG CCG CGC AGC CTC CGC CTG GCG CTG GCG CTA CGC | 743 |
| Asn Ala Ser Trp Pro Arg Ser Leu Arg Leu Ala Leu Ala Leu Arg | |
| 210 215 220 | |
| CCC CGG GCC CCT GCC GCC TGC GCG CGC CTG GCC GAG GCC TCG CTG | 788 |
| Pro Arg Ala Pro Ala Ala Cys Ala Arg Leu Ala Glu Ala Ser Leu | |
| 225 230 235 | |
| CTG CTG GTG ACC CTC GAC CCG CGC CTG TGC CAC CCC CTG GCC CGG | 833 |
| Leu Leu Val Thr Leu Asp Pro Arg Leu Cys His Pro Leu Ala Arg | |
| 240 245 250 | |

| | |
|--|------|
| CCG CGG CGC GAC GCC GAA CCC GTG TTG GGC GGC GGC CCC GGG GGC | 878 |
| Pro Arg Arg Asp Ala Glu Pro Val Leu Gly Gly Gly Pro Gly Gly | |
| 255 260 265 | |
| GCT TGT CGC GCG CGG CGG CTG TAC GTG AGC TTC CGC CAG GTG GGC | 923 |
| Ala Cys Arg Ala Arg Arg Leu Tyr Val Ser Phe Arg Glu Val Gly | |
| 270 275 280 | |
| TGG CAC CGC TGG GTC ATC GCG CCG CGC CCC TTC CTG GCC AAC TAC | 968 |
| Trp His Arg Trp Val Ile Arg Pro Arg Gly Phe Leu Ala Asn Tyr | |
| 285 290 295 | |
| TGC CAG GGT CAG TGC GCG CTG CCC GTC GCG CTG TCG GGG TCC GGG | 1013 |
| Cys Gln Gly Gln Cys Ala Leu Pro Val Ala Leu Ser Gly Ser Gly | |
| 300 305 310 | |
| GGG CCG CCG GCG CTC AAC CAC GCT GTG CTG CGC GCG CTC ATG CAC | 1058 |
| Gly Pro Pro Ala Leu Asn His Ala Val Leu Arg Ala Leu Met His | |
| 315 320 325 | |
| GCG GCC GCC CCG GGA GCC GCC GAC CTG CCC TGC TGC GTG CCC GCG | 1103 |
| Ala Ala Ala Pro Gly Ala Ala Asp Leu Pro Cys Cys Val Pro Ala | |
| 330 335 340 | |
| CGC CTG TCG CCC ATC TCC GTG CTC TTC TTT GAC AAC AGC GAC AAC | 1148 |
| Arg Leu Ser Pro Ile Ser Val Leu Phe Phe Asp Asn Ser Asp Asn | |
| 345 350 355 | |
| GTG GTG CTG CGG CAG TAT GAG GAC ATG GTG GTG GAC GAG TGC GGC | 1193 |
| Val Val Leu Arg Gln Tyr Glu Asp Met Val Val Asp Glu Cys Gly | |
| 360 365 370 | |
| TGC CGC TAACCCGGGG CGGGCAGGGA CCCGGGCCCA ACAATAAATG CCGCGTGG | 1238 |
| Cys Arg | |
| 372 | |

(34) INFORMATION FOR SEQ ID NO:33:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 372 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 (A) ORGANISM: human
 (F) TISSUE TYPE: BRAIN
- (ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION:
 (D) OTHER INFORMATION: /function=
 /product= "GDF-1"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

| | | | | | | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|--|--|--|--|--|
| | Met | Pro | Pro | Pro | Gln | Gln | Gly | Pro | Cys | Gly | | | | | | | | | | |
| | 1 | | | | 5 | | | | | 10 | | | | | | | | | | |
| His | His | Leu | Leu | Leu | Leu | Leu | Ala | Leu | Leu | Leu | Pro | Ser | Leu | Pro | | | | | | |
| | | | | 15 | | | | | 20 | | | | | 25 | | | | | | |
| Leu | Thr | Arg | Ala | Pro | Val | Pro | Pro | Gly | Pro | Ala | Ala | Ala | Leu | Leu | | | | | | |
| | | | | 30 | | | | | 35 | | | | | 40 | | | | | | |
| Gln | Ala | Leu | Gly | Leu | Arg | Asp | Glu | Pro | Gln | Gly | Ala | Pro | Arg | Leu | | | | | | |
| | | | | 45 | | | | | 50 | | | | | 55 | | | | | | |
| Arg | Pro | Val | Pro | Pro | Val | Met | Trp | Arg | Leu | Phe | Arg | Arg | Arg | Asp | | | | | | |
| | | | | 60 | | | | | 65 | | | | | 70 | | | | | | |
| Pro | Gln | Glu | Thr | Arg | Ser | Gly | Ser | Arg | Arg | Thr | Ser | Pro | Gly | Val | | | | | | |
| | | | | 75 | | | | | 80 | | | | | 85 | | | | | | |
| Thr | Leu | Gln | Pro | Cys | His | Val | Glu | Glu | Leu | Gly | Val | Ala | Gly | Asn | | | | | | |
| | | | | 90 | | | | | 95 | | | | | 100 | | | | | | |
| Ile | Val | Arg | His | Ile | Pro | Asp | Arg | Gly | Ala | Pro | Thr | Arg | Ala | Ser | | | | | | |
| | | | | 105 | | | | | 110 | | | | | 115 | | | | | | |

| | | | | | |
|-----------------|---------------------|-------------------------|-----|-----|-----|
| Glu Pro Val Ser | Ala Ala Gly His Cys | Pro Glu Trp Thr Val Val | 120 | 125 | 130 |
| Phe Asp Leu Ser | Ala Val Glu Pro Ala | Glu Arg Pro Ser Arg Ala | 135 | 140 | 145 |
| Arg Leu Glu Leu | Arg Phe Ala Ala Ala | Ala Ala Ala Ala Pro Glu | 150 | 155 | 160 |
| Gly Gly Trp Glu | Leu Ser Val Ala Gln | Ala Gly Gln Gly Ala Gly | 165 | 170 | 175 |
| Ala Asp Pro Gly | Pro Val Leu Leu Arg | Gln Leu Val Pro Ala Leu | 180 | 185 | 190 |
| Gly Pro Pro Val | Arg Ala Glu Leu Leu | Gly Ala Ala Trp Ala Arg | 195 | 200 | 205 |
| Asn Ala Ser Trp | Pro Arg Ser Leu Arg | Leu Ala Leu Ala Leu Arg | 210 | 215 | 220 |
| Pro Arg Ala Pro | Ala Ala Cys Ala Arg | Leu Ala Glu Ala Ser Leu | 225 | 230 | 235 |
| Leu Leu Val Thr | Leu Asp Pro Arg Leu | Cys His Pro Leu Ala Arg | 240 | 245 | 250 |
| Pro Arg Arg Asp | Ala Glu Pro Val Leu | Gly Gly Gly Pro Gly Gly | 255 | 260 | 265 |
| Ala Cys Arg Ala | Arg Arg Leu Tyr Val | Ser Phe Arg Glu Val Gly | 270 | 275 | 280 |
| Trp His Arg Trp | Val Ile Arg Pro Arg | Gly Phe Leu Ala Asn Tyr | 285 | 290 | 295 |
| Cys Gln Gly Gln | Cys Ala Leu Pro Val | Ala Leu Ser Gly Ser Gly | 300 | 305 | 310 |
| Gly Pro Pro Ala | Leu Asn His Ala Val | Leu Arg Ala Leu Met His | 315 | 320 | 325 |
| Ala Ala Ala Pro | Gly Ala Ala Asp Leu | Pro Cys Cys Val Pro Ala | 330 | 335 | 340 |
| Arg Leu Ser Pro | Ile Ser Val Leu Phe | Phe Asp Asn Ser Asp Asn | 345 | 350 | 355 |
| Val Val Leu Arg | Gln Tyr Glu Asp Met | Val Val Asp Glu Cys Gly | 360 | 365 | 370 |
| Cys Arg | | | 372 | | |

What is claimed is:

1. A method of screening candidate compounds for the ability to modulate the effective concentration of a morphogen in an organism, said method comprising
incubating a candidate compound with cells from a test tissue type known to produce a morphogen for a time sufficient to allow said compound to affect the production of said morphogen, and
assaying said cells for a parameter indicative of a change in the level of production of said morphogen.
2. The method of claim 1 wherein said morphogen is OP-1.
3. The method of claim 2 wherein said test tissue type is a human renal-derived tissue.
4. The method of claim 3 wherein said renal-derived tissue is a kidney or bladder-derived tissue.
5. The method of claim 2 wherein said test tissue type is adrenal-derived tissue.
6. The method of claim 1 wherein said morphogen is GDF-1.
7. The method of claim 6 wherein said test tissue type is derived from human nerve tissue.

8. The method of claim 7 wherein said nerve tissue is brain-derived tissue.

9. The method of claim 1 wherein said morphogen is DPP.

10. The method of claim 9 wherein said test tissue type is derived from one of the following drosophila tissues: dorsal ectoderm, epithelial imaginal disc visceral mesoderm, or gut endoderm.

11. The method of claim 1 wherein said morphogen is Vgr-1.

12. The method of claim 11 wherein said test tissue type is mouse lung tissue.

13. The method of claim 1 wherein said morphogen is Vgl.

14. The method of claim 13 wherein said test tissue type is xenopus fetal endoderm tissue.

15. A method of assessing a tissue of an organism for its level of production of a morphogen and for screening candidate compounds for the ability to modulate the effective concentration of said morphogen produced by cells of said tissue, said method comprising
selecting a test tissue type producing a high level of morphogen relative to the level of morphogen produced by other tissue types;

incubating a candidate compound with cultured cells of said selected tissue type for a time sufficient to allow said compound to affect the production of said morphogen; and

assaying said selected tissue cells for a parameter indicative of a change in the level of production of said morphogen.

16. The method of claim 1 or 15 wherein said parameter indicative of the level of said morphogen is determined using an antibody specific for said morphogen.

17. The method of claim 1 or 15 wherein said parameter indicative of the level of said morphogen is determined by measuring cellular proliferation in cells which are sensitive to the concentration of secreted OP-1.

18. The method of claim 1 or 15 wherein said parameter indicative of the level of said morphogen is determined using a nucleic acid probe that hybridizes under stringent conditions with nucleic acid encoding said morphogen.

19. The method of claim 18 wherein said morphogen comprises a minimally active core C-terminal region comprising at least six cysteine residues, and said nucleic acid probe hybridizes with an mRNA encoding a region N-terminal to said core region.

20. The method of claim 18 wherein said morphogen comprises a minimally active core C-terminal region

comprising at least six cysteine residues, and said nucleic acid probe hybridizes with an mRNA encoding a region 3' to said core region.

1 / 3

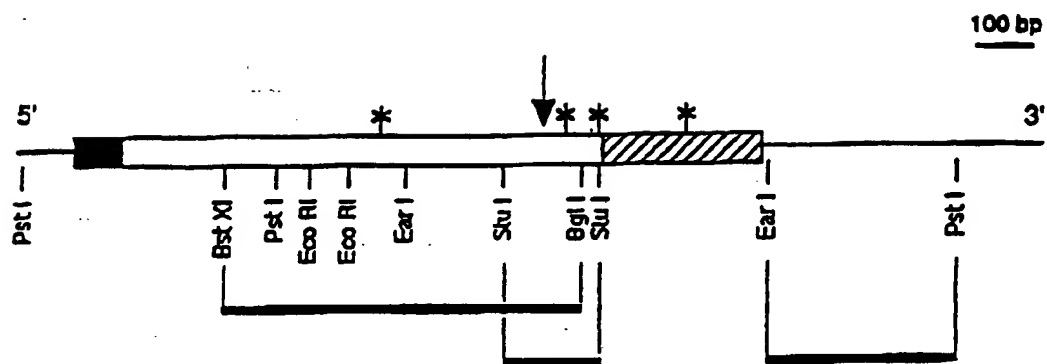


Fig 1

2 / 3

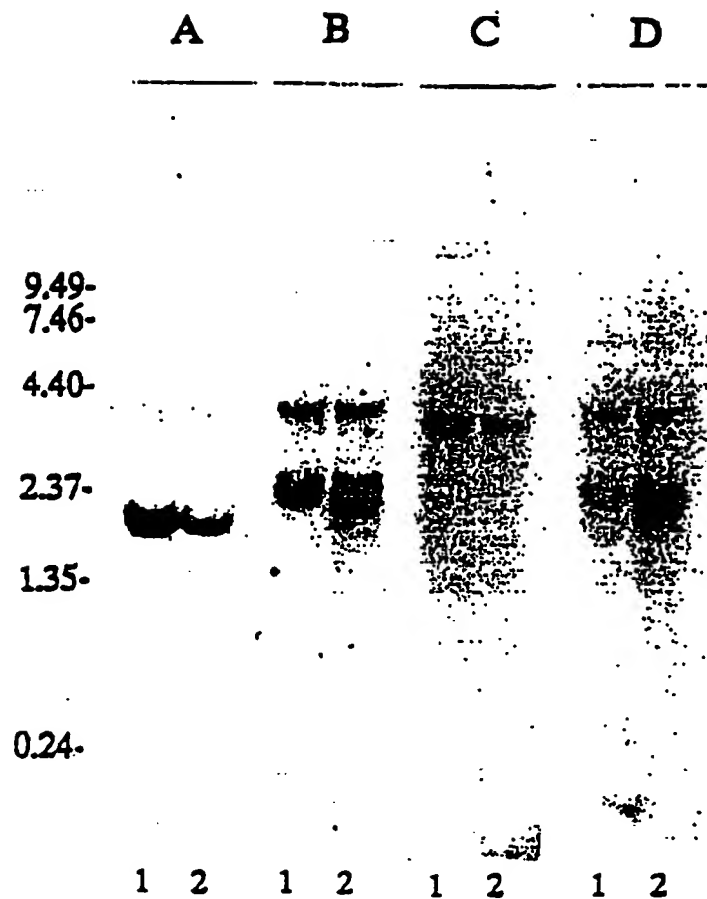


Fig 2

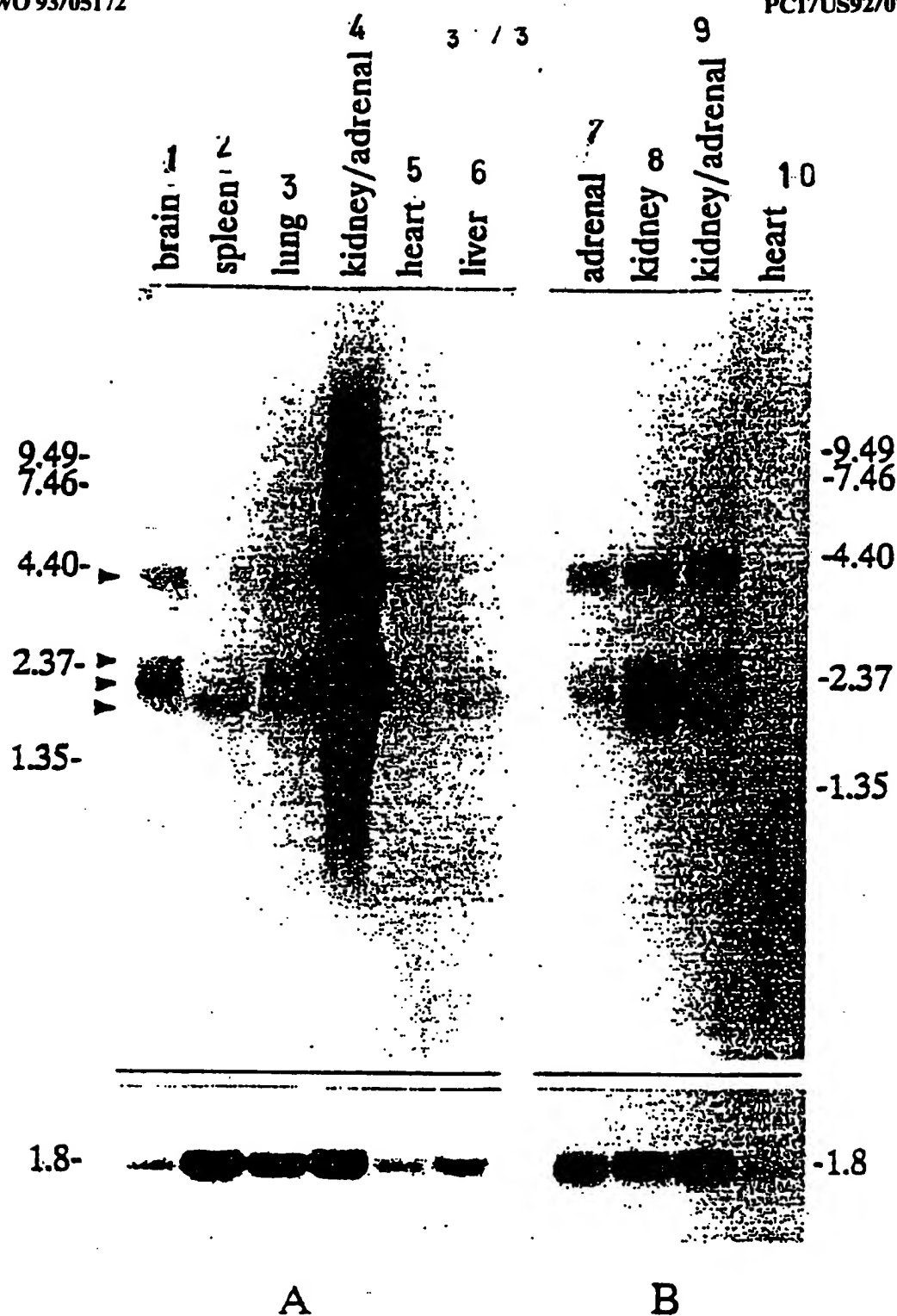


Fig 3

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 92/07359

| | | |
|---|---|-------------------------------------|
| I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) ⁶ | | |
| According to International Patent Classification (IPC) or to both National Classification and IPC | | |
| Int.Cl. 5 C12Q1/02; G01N33/68 | | |
| II. FIELDS SEARCHED | | |
| Minimum Documentation Searched ⁷ | | |
| Classification System | Classification Symbols | |
| Int.Cl. 5 | C12Q ; G01N ; C07K | |
| Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁸ | | |
| III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹ | | |
| Category ¹⁰ | Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹² | Relevant to Claim No. ¹³ |
| X | JOURNAL OF BONE AND MINERAL RESEARCH vol. 6, no. 7, July 1991, pages 767 - 777 H.ZHOU ET AL. | 1, 15, 18 |
| Y | see abstract see page 768, left column, line 22 - line 55 see page 771, right column, line 12 - page 772, left column, line 8 see page 774, right column, line 4 - line 44 --- -/- | 6 |
| <p>¹⁰ Special categories of cited documents:</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principles or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"A" document member of the same patent family</p> | | |
| IV. CERTIFICATION | | |
| Date of the Actual Completion of the International Search | Date of Mailing of this International Search Report | |
| 09 DECEMBER 1992 | 13. 01. 93 | |
| International Searching Authority | Signature of Authorized Officer | |
| EUROPEAN PATENT OFFICE | LUZZATTO E.R. | |

| III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET) | | |
|--|---|-----------------------|
| Category * | Citation of Document, with indication, where appropriate, of the relevant passages | Relevant to Claim No. |
| X | <p>PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA. vol. 86, June 1989, WASHINGTON US pages 4554 - 4558 K. LYONS ET AL. cited in the application see abstract see page 4557, left column, line 34 - page 4558, line 18; figure 5</p> | 1, 11, 15, 19 |
| X | <p>WO, A, 9 102 744 (CELTRIX LABORATORIES) 7 March 1991 see page 1, line 1 - page 3, line 34 see page 29, line 1 - line 28</p> | 15, 16 |
| Y | <p>PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA. vol. 88, May 1991, WASHINGTON US pages 4250 - 4254 S.-J. LEE see abstract</p> | 6 |
| Y | <p>WO, A, 9 000 619 (UNIVERSITY COLLEGE LONDON) 25 January 1990 see page 1, line 1 - page 2, line 18 see page 4, line 14 - page 14, line 10</p> | 1, 15 |
| P, Y | <p>BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS. vol. 179, no. 1, 30 August 1991, DULUTH, MINNESOTA US pages 116 - 123 E. ÖZKAYANAK ET AL. see the whole document</p> | 1, 15 |

**ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO. US 9207359
SA 64596**

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information. 09/12/92

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---------------------|----------------------------|---------------------|
| WO-A-9102744 | 07-03-91 | AU-A- 6187090 | 03-04-91 |
| | | CA-A- 2064878 | 22-02-91 |
| | | EP-A- 0489062 | 10-06-92 |
| <hr/> | | | |
| WO-A-9000619 | 25-01-90 | JP-T- 3505669 | 12-12-91 |
| <hr/> | | | |